ECONOMIC AND ENGINEERING ASPECTS OF DISPOSABLES-BASED BIOPROCESSING

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by

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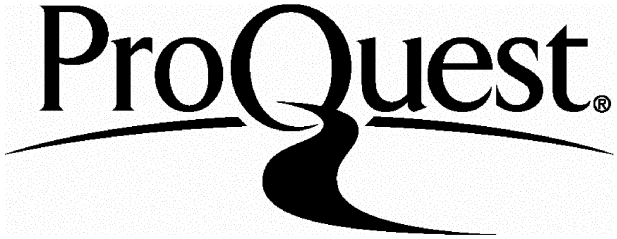

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ABSTRACT

Biopharmaceutical companies face a fast moving competitive market with high product failure rates. Disposables-based bioprocessing meets some of these current pressures and concerns by employing single-use, pre-sterilised, pre-validated components instead of traditional stainless-steel fixed equipment. The advantages include increased flexibility, smaller initial investments and potential reduction of time to market. This thesis provides an engineering study of the use of disposable equipment as an alternative to conventional systems.

A costing framework was developed to compare disposables-based and conventional plants. The use of disposable equipment was shown to result in a 70% increase in running costs, substantially offset by a 40% reduction in the capital investment required. The production of a Fab' antibody fragment from an *E. coli* fermentation was used as the illustrative case study. Sensitivity analysis to different variables was made to confirm the results. The study showed a loss in yield in different unit operations in the disposable process could be compensated for by a reduction of the materials costs. It was also predicted that the use of disposables could reduce time to market by up to 1.5 years.

The running costs associated with the single use of microfiltration membranes were shown to have a high impact on the overall cost indicating that minimisation of membrane area was crucial. Experimental work focused on this unit operation, aimed at controlling transmission. Transmission was shown to decrease rapidly and the causes for this decrease were explained and modelled. A strategy that maintains % transmission at high values was developed and evaluated theoretically and experimentally. The method comprises short intermediate rinsing steps, capable of restoring the membrane properties. The resulting reduced filtration areas were shown to enhance further the economic attractiveness of the disposable approach.

Overall disposables-based bioprocessing was shown to be economically and technologically competitive with conventional engineering approaches.

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ABBREVIATONS

A/E	architectural/engineering
BSA	bovine serum albumin
cGMP	current good manufacturing practices
CIP	clean-in-place
CV	column volume
DCW	dry cell weight
DEAE	diethylaminoethyl
DOT	dissolved oxygen tension
DNA	desoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme linked immunoabsorbant assay
EPCM	engineering procurement and construction management
FCI	fixed capital investment
FDA	US Food and Drug Administration
HPTFF	high performance tangential flow filtration
HSA	human serum albumin
HVAC	heating, ventilation and air conditioning
IPTG	isopropyl-β-D-thiogalactopyranoside
IQ	installation qualification
IR	infra red
MF	microfiltration
MWCO	molecular weight cut-off
NPV	net present value
NWP	normalised water permeability
OD	optical density
OL	operating labour
OQ	operational qualification
PBS	phosphate buffered saline
PES	polyethersulfone
PPG	polypropylene glycol
PQ	performance qualification
PVDF	polyvinylidene fluoride

PW	purified water
QA	quality assurance
QC	quality control
R&D	research and development
RO	reverse osmosis
SOP	standard operating procedure
TFF	tangential flow filtration
TMP	transmembrane pressure
tPA	tissue plasminogen activator
UF	ultrafiltration
UV	ultra violet
WFI	water for injection

Chapter 1 General introduction

1.1 Introduction

This thesis examines the use of disposable equipment as a novel approach to biopharmaceutical production. The trend to this new solution is mainly driven by today's market, where flexibility and speed are key issues. Flexibility is crucial in biopharmaceutical manufacturing due to the high level of new drugs failures during clinical trials. Speed to market is essential to maximise revenue during patent life (Dunnill and Davies, 1998).

In order to determine the importance and interest of such a technological approach the first aim of this thesis was to evaluate disposables-based processes from an economic point of view and to compare these with traditional bioprocessing methods (Part I: Chapters 2 to 4). The running costs associated with the single use of membranes employed in tangential flow filtration steps are shown to have a significant impact on the overall cost indicating a need for minimisation of membrane areas. The second aim and part of this work has consequently been to investigate experimentally disposable membrane separation, the main objective being to reduce the filtration areas required whilst achieving acceptable levels of process performance (Chapters 5 to 8). Finally overall conclusions were drawn from both parts of the thesis as presented in Chapter 9 followed by suggestions of future work (Chapter 10). Appendices 1 and 2 present basic data and calculations used to support the main thesis chapters. Additionally an analysis of the potential commercial exploitation of the disposables concept is presented in Appendix 3. The executive summary and business plan were prepared as part of a New Venture Development course, attended at London Business School

1.2 Market overview

The enormous cost of R&D in the biopharmaceutical industry derives from long development time lines, typically 5 to 12 years with an average of 7.8 years (Hamers, 1993; Foo et al., 2001), and high product failure rates together with the need to evaluate large product portfolios in order to obtain successful candidates (only 23% of drugs entering Phase I clinical trials get to market, Breggar, 1996).

Today it is no longer sufficient to have a good technology portfolio, a strong intellectual property position and access to capital (Gamerman and Mackler, 1994). Flexibility, time to market and cost effectiveness are becoming the key issues with which biopharmaceutical companies have to be concerned (Gamerman and Mackler, 1994; Basu, et al., 1998; Burnett, et al., 1991; Ernst, et al, 1997; Hamers, 1993).

This scenario is likely to become emphasised with the advent of new potential drug candidates deriving from the decoding of the human genome. The key to success is production flexibility, which is dictated both by constant priority changes arising from the generation of safety and clinical data in the development phase (Basu, et al., 1998) and by the need to allow for future expansions. As a consequence of priority changes, the capability of multiproduct processing is gaining an increasing interest although there are still complicated regulatory issues associated with potential cross-contamination (Hamers, 1993). Multiproduct processing is not easily achieved in conventional stainless-steel plants resulting in precious time and resources being spent to avoid and validate the absence of cross-contamination, which can represent 15% of the total risks (Stedim corporate profile, 2000; Joly, 1998). Future process volumes need to be carefully considered during the design of any facility. The problem is that when design decisions are made early in the development process they are difficult to change later due to regulatory constraints (Basu, et al., 1998; Ernst, et al, 1997). On the other hand, the delay of the decision to build brings construction onto the critical path (Nicholson, 1998). Early decision-making would be advantageous but will be associated with higher risk since there is less confidence in the likelihood of success of the product (Burnett, et al., 1991). This is particularly critical for biopharmaceutical products due to their high failure rates. Ward (2000) considers that the best strategy to minimize risk is to delay expenditure and to minimize project cost, as well as to design an adaptable plant.

Secondly, it is essential to get into the market as quickly as possible due to increasing competitiveness (Burnett, et al., 1991) and to maximise revenue during patent life. Indeed the long development times significantly cut into the 20 years patented lifetime. In order to cut healthcare budgets generic drugs are being favoured and newly released drugs no longer command large premiums (Nicholson, 1998). Companies must therefore move more quickly from discovery to patent, and then to trials and efficient

production. According to Basu, et al. (1998) delays in entering the market translate into millions of dollars of lost revenue. Time to market is often the key to economic success (Cooney, 1995).

One immediate problem facing the biotechnology industry is a severe shortage of manufacturing capacity for the new drugs that will soon be approved (Garber, 2001). In particular at the time of writing this thesis contract manufacturers are fully booked with waiting lists of 1 to 2 years. This wait adds further to the already long development times.

The advent of "generics" to replace existing drugs as they come off patent is a very important issue in the pharmaceutical industry today, as are safer, more efficacious and easier to deliver drugs that constantly displace earlier drugs from the market. This means that revenue generated during the patent life is crucial and must be maximised through gaining rapid market entry.

Finally, the industry must operate under growing government- and market-enforced price controls and a need for cost-benefit justification (Gamerman and Mackler, 1994) which brings a demand for better cost effectiveness. Additionally there is less confidence on the part of the investors and a lack of available capital which forces companies to control their capital needs. According to Cooney (1995) cost effectiveness is a constraint on many therapeutic opportunities. Indeed less than 30% of drugs marketed between 1980 and 1984 produced revenues that matched or exceeded average R&D costs (Grabowski and Vernon, 1994).

The problem today is that governments and insurance agencies alike have to face ageing populations with growing health care needs. In order to cut healthcare budgets generic drugs are being favoured, as well as there is a trend back to cheaper, older drugs (Dunnill and Davies, 1998). Newly released drugs no longer command large premiums. Companies must therefore move more quickly from discovery to patent and to trials and efficient production. The production of material for trials (materialisation) lies more often on the critical path as organisational, regulatory and scientific approaches decrease the time for establishing efficiency.

The next section will analyse the features and limitations of conventional bioprocessing plants to meet the market demands outlined above. A section describing fully disposable plants as an alternative to conventional designs will then follow.

1.3 Conventional stainless-steel bioprocessing plants

Conventional biopharmaceutical processing facilities are based on the heavy use of stainless steel equipment interconnected by stainless steel pipes. These plants also include a variety of utilities such as steam production and clean-in-place (CIP) together with the connecting lines to achieve cleaning and sterilisation between subsequent batches or continuous production periods.

On the one hand stainless steel equipment is very expensive which makes it difficult for companies to make improvements in the process or the products due to the high capital costs involved. This is also a problem for companies that are developing a new product due to the high failure rates of potential new products during the clinical development phases, and also because of the long critical time elapsed between clinical trials and operation at production scale. Stainless steel plants are also very inflexible with regard to the need of companies to take a number of candidates through development in overlapping schedules.

Finally the resultant cost of down time for cleaning, sterilisation and validation procedures as well as labour and operating costs associated to these operations reduce still further the competitiveness of stainless steel plants. Moreover, any cleaning procedure has to be validated and this accounts for man work costs, consumables costs and down time costs.

1.4 Disposables-based bioprocessing plants

1.4.1 Description

Disposables-based technology makes use of fully disposable equipment as an alternative to conventional systems. In such a re-engineering stainless steel vessels would be replaced by rigid plastic containers containing pre-sterilised disposable plastic bags requiring virtually no maintenance and incurring minimal cleaning costs. These bags meet the biocompatibility requirements of biopharmaceutical applications

(Joly, 1998). The same concept applies to the connections, which can be replaced by disposable plastic pipes. This option has therefore the advantage of switching capital costs to consumables costs as required. It also allows for the better management of uncertainty in the planning of future process volumes.

One major challenge to be encountered when opting for disposables is mechanical agitation, which is crucial in a fermenter. Alternative designs have to be considered such as airlift or the use of a plunging jet design, which consists on an external pumping loop to achieve mixing and gas mass transfer (Murrell et al., 2000). Work performed at UCL with such a design has indicated that a yield of 67% of that obtainable with a stirred tank can be reached. The lower performance is due to oxygen transfer limitations (Baker, 2001).

For mammalian cell culture there is currently a disposable bioreactor design based on wave-induced agitiation developed by Wave Biotech (Bedminster, NJ, USA). The limitations of this technology reside mainly on scalability and the fact that the design would not be suitable for *E. coli* due to the high oxygen and mixing requirements of bacterial fermentations.

The disposable concept can be extended throughout the production process. Separation processes such as cell harvesting and protein clarification and concentration can be achieved by tangential flow filtration with disposable membranes. Some companies have now developed intrinsically disposable membranes (e.g. Spectrum Laboratories), which are substantially cheaper than conventional ones (see Chapter 4). There is also a trend to adapt components from existing technologies such as the use of kidney dialysis cartridges for animal cell culture for small scale production of monoclonal antibodies (Marx et al. 1997). The advantage of this practice is that due to the large main market the cartridges are sufficiently cheap as to be disposed of after use. Where a disposable membrane design is not available however an effort directed towards the minimization of the filtration area will have to be made in order to cut the costs associated with these steps. Part II of this thesis will address this issue.

The final purification steps can be accomplished in disposable pre-packed chromatography columns or by batch adsorption in disposable plastic bags. The main

difficulty will possibly be to avoid affinity chromatography, in which case the single use of the currently expensive matrix would become a major cost concern.

Disposable technology also makes use of non-invasive pumps and valves, such as peristaltic pumps and pinch valves. All the instrumentation is disposable or non-invasive and heat transfer can be achieved by disposable heat-exchangers. A patent filed by Pearl and Christy (2000) describes a heat exchanger with an operating concept similar to that of flat-sheet tangential flow membranes, but with a heat exchanging surface replacing the membrane.

Although not traditionally disposable, some disposable applications of centrifugation have been developed. For example Sorvall (Kendro) has developed a large capacity separation system (Centritech R cell), in which the process material only comes in contact with a sterile disposable liner. According to the manufacturer this device can be used for cell harvesting of mammalian and insect cells.

Instrumentation has to be either non-invasive, such as UV detectors and gas mass spectrometers, or disposable. For example thermocouples can be sufficiently cheap to be used only once. Another alternative could be the measurement of the outside surface temperature, if a correlation with the vessel temperature can be found. Again, the medical device industry can be a source of disposable apparatus, like for example pressure transducers used for blood pressure monitoring (e.g. Deltran® from Utah Medical Products, Inc.). Pall Corporation has also developed disposable pressure transducers for membrane filtration applications (Sellick, 2000). These devices withstand pressures of up to 45 psi and should cost approximately $300 per 12-pack. Pall is also developing disposable flowmeters for the same application, based on a spiral path and an IR detector. In this case there is a fixed part costing around $200 and a disposable part for approximately $30. pH and DOT are examples of properties that may be difficult to measure in a disposable or non-invasive way. Murrell et al. (2000) suggest alternatives such as the use of fibre optics or an external autoclaved loop. Data interpretation from actual available measurements (e.g. cell density by optical window, exit gas analysis, etc) can also be the solution in some cases.

Table 1.1 summarises the key features of a disposables-based facility. Despite the differences from a conventional approach such a facility would nevertheless be

compliant with the requirements of cGMP (current good manufacturing practices) for biopharmaceutical products.

Disposable technology can be used both for the process development stages and for manufacture, provided the process does not require large volumes, which is still the case for many biotech drugs. Indeed the fermentation maximum volume would be 2500 L, set by the maximum size currently available for disposable process bags (Stedim S.A., France; Hyclone Laboratories, Inc., UT, USA). Nonetheless transition to a conventional process at the manufacturing stage is easily achieved since the disposable processes are compatible with a stainless steel design.

CONVENTIONAL	DISPOSABLE
• stainless steel vessels	• disposable bags
• stirred tank fermenter	• bag with recirculation loop to promote mixing
• stainless steel piping	• flexible, disposable tubing
• valves, pumps, connections	• pinch valves, peristaltic pumps, sterile welding
• membranes, centrifuges	• disposable membranes (or disposable centrifuges)
• chromatography columns	• batch adsorption or pre-packed columns
• media and buffers preparation tanks	• ready-made, pre-sterilised media and buffers bought in bags
• heat transfer devices	• recirculation loop with disposable heat exchanger
• pressure, temperature, pH probes	• disposable or non-invasive probes
• additions	• sterile welded connections
• sampling	• needleless syringes
• utilities	• generally reduced: - no CIP/steam facilities required - WFI/Purified water may be bought in disposable containers
• waste treatment	• reduced liquid effluents, increased solids for disposal

Table 1.1 Features of conventional bioprocessing plants and corresponding disposables-based solution.

1.4.2 Advantages

The disposables-based approach to bioprocessing will help solve some of the serious current concerns outlined in the introduction. Table 1.2 and its discussion below help clarify how this can be achieved.

As a disposables-based production train can be put together at low cost, a delay in the sanctioning of large capital investments is possible. Such a capability is crucial to reduce the risk in the early process development stages where uncertainty about the success of the product is extremely high. Also, modifications in process steps or process volumes are more straightforward and achievable with smaller capital expenditure. In-house manufacture can become accessible to companies that could not otherwise afford to build their own facility. A further major perceived advantage of this technology is that for small new companies entry to expensive clinical trials manufacture is at a reduced cost and hence at a lower risk.

A key factor determining the speed to market of disposables-based plants is associated with the decision of when to build the manufacturing facility. The simpler construction of disposables-based plants implies that shorter implementation times can be realised which allows for more detailed process optimisation before moving onto construction. Alternatively, these shorter construction times may allow for earlier entry to market and at a lower risk due to the smaller investment involved. This is possibly one of the most important achievements of the use of disposables. Additionally the easier changeover allows a higher throughput of drug candidates. The concept "fail fast, fail cheap" (Rosenberg, 2000) becomes a reality. Furthermore an early entry to market increases the exploitable patent life and generates a stronger position in the market.

The short implementation times could also constitute a solution to the current problem of lack of capacity, with contract manufacturers choosing to build extra capacity based on disposable equipment.

The employment of single-use, pre-sterilised equipment eliminates the problems associated with clean-in-place and sterilisation between successive batches or different products. The validation procedures as well as labour and operating costs associated with these operations are also minimized. In Lonza Biologics' experience (Bevan, 2000) bags with integral sterile filters are more cost effective than stainless steel at

small to medium scale due the absence of steam sterilisation. Adner and Sofer (1994) report that cleaning and validation of cleaning are among the most critical issues in biopharmaceutical processes. In particular cleaning represents on average 20% of the chromatography cycle time. The use of disposables therefore also results in a reduction of down-time and turn-around time, as the only operations required with this approach are disassembling/reassembling of the disposable items. According to Monge (1996), turnaround times with this technology can be reduced by up to 2 months per year. One direct consequence may be an increase in productivity, as the use of disposables improves the plant "up time" and consequently also the lot frequency.

Despite the disappointingly small number of genes discovered in the human genome, it is estimated that the search for disease genes can now be carried out in a matter of months (Bailey et al., 2001). This will lead to an increase in the number of small scale, personalised (orphan) drugs. The use of disposables may help to improve the economic interest of such drugs: despite the low volumes required a process can be put together with a low investment and easily changed over to a different product.

Biotech needs	**Disposable plant capabilities**
• minimise capital risk	• equipment costs shifted to running costs as required (reduced capital expenditure)
• increased flexibility	• simplified turn-around between different products • process changes/expansions at a minimal capital cost
• compression of development time-scales	• shorter down-time and turn-around time • reduced validation • plant construction out of the critical path

Table 1.2 Summary of the capabilities of disposables-based plants.

1.4.3 Issues arising from the use of disposable equipment

Some difficulties with the regulatory authorities can be anticipated over the acceptance of disposables-based plants. In addition to normal validation there will be a need to validate the assembly of the different components before each batch, thus leading to

the need to establish standard operation procedures (SOP's) for each connection and installation. There is however a strong indication that this will not be a major obstacle as some companies already make use of disposable components in parts of their processes (see section 1.4.4).

The opposition of ecological associations may also be perceived as a possible setback, due to the generation of more solid waste. This may be partially overcome by the recycling of certain components, if contamination issues can be overcome. The reduction of effluents due to the absence of CIP may also balance the increase in solid residues (possibly including bagged waste).

Finally disposables-based plants must be easy to scale-up in order that shorter implementation times can be realised. Most importantly there will be a need to validate the transition from a disposable process into a conventional stainless steel process where the commercial scale cannot be met by disposables. The major difficulty will be to obtain FDA approval for a transition at late stage clinical trials. It will therefore be of major importance to develop disposable unit operations that are as close as possible to their conventional equivalents. Alternatively consistent rules for the translation across technologies will have to be developed.

1.4.4 Current uses of disposables

For economic reasons some biotechnology companies (most notably in the US) have had to introduce changes in their processes which can be seen as a first step towards disposable technology. These changes include the use of flexible plastic lines instead of stainless steel, and sterile connections instead of valves. Companies such as Lonza Biologics use some disposable components such as media bags and some of their pumps are non-invasive. Also some companies buy water for injection (WFI) containers instead of having their own purification facility.

Flexible tissue culture bags were developed in the mid-1980s to replace traditional cell culture techniques for human therapy in an attempt to render them more reliable and reproducible (Armstrong et al, 1995). Disposable bags and liners for rigid containers are now available at up to 2500L scale (Stedim corporate profile, 2000). These are however only still used for the supply of feed-stock and collection of product.

Merck, amongst other companies, no longer reuse the membranes used in separation applications, due to the high cost and difficulty of the cleaning step validation (van Horn, 1998; Meacle et al., 1999).

An example of evolution towards flexibility is the Ares-Serono biotechnology plant that has production areas that can be easily and quickly adapted to produce different quantities of any product through the use of movable skids according to market demand (Anon., 1996). This concept can be very suitable for disposables-based processes, where the bags not in use can be stored in a collapsed shape therefore taking up little space.

The evaluation of disposables-based mode of operation will not be complete without performing economic viability studies with the models described in the following section.

1.5 Economic modelling

Until recently biopharmaceutical drugs were very expensive drugs made on small scales with high pricing flexibility, which meant that economic aspects of projects were not as crucial as they are today. For that reason, and despite the dissimilarities, chemical engineering models have been widely used to evaluate biopharmaceutical projects, without major modifications (Atkinson and Mavituna, 1991; Bailey and Ollis, 1986; Reisman, 1988; Mathys et al., 1999; Petrides et al., 1995). A limited amount of biochemical engineering data is also available through papers based on personal experience or actual projects (Datar and Rosen, 1990; Datar et al., 1993; Nizel and Schoenfeld, 1996; Beck, 2000; Ward, 2000). A thorough review of economic models for the calculation of capital investment and running costs of biopharmaceutical plants will be presented in Chapter 2. These costs can then be used to calculate the net present value (or net present worth), which gives an indication of the profitability of a project.

The net present value (NPV) works by comparing the difference between the present value of cash flow generated by future product sales with the present value of the investment (Nicholson and Latham, 1994). The generic equation for its calculation is (adapted from Peters and Timmerhaus, 1991):

$$NPV = \sum_{n=0}^{m+\tau} \frac{CF_n - FCI_n}{(1+r)^n} = \sum_{n=0}^{m+\tau} \frac{S_n - RC_n - FCI_n}{(1+r)^n}$$

Equation 1.1

where NPV is the net present value of the project, r is the discount rate (or annual interest rate of return), CF_n is the net cash flow in year n, FCI_n is the fixed capital investment in year n, τ is the life of the project (in years), m is the year of entry to market, S_n is the value of sales in year n and RC_n is the value of the running costs in year n. For $n < m$, i.e. before entry to market, S_n will be zero and Eq. 5 becomes:

$$NPV = \sum_{n=0}^{m-1} \frac{-RC_n - FCI_n}{(1+r)^n} + \sum_{n=m}^{m+\tau} \frac{S_n - RC_n - FCI_n}{(1+r)^n}$$

Equation 1.2

with the first part of the equation dealing with the period before manufacture commences and the second part with the period thereafter.

The fixed capital investment is a one-off cost that must be supplied up-front in order to purchase, build, install and validate the necessary machinery, equipment and buildings (Peters and Timmerhaus, 1991). Running costs are on-going costs associated with the operation of the plant. Models for the evaluation of these two types of costs in biochemical engineering plants are reviewed in Chapter 2. Most importantly these models will be modified in Chapter 3 to accommodate features specific to disposables-based operation.

Sensitivity analysis is necessary since the economic analysis of a project relies strongly on estimates. This allows the evaluation of the impact of key uncertainties.

Other economic indicators, not used in this work due to their limitations, include:

- Internal rate of return, the discount rate (r) for which the NPV of the project is zero (Osborne, 1998). This value can be compared to a criterion rate to see if the project is an attractive investment.

- Pay back time, which indicates the time required to pay off the initial investment (Sinnott, 1991). This indicator does not give any information on the performance of the project after the pay back period.

- Rate of return, defined as the ratio of the cumulative net cash flow at the end of the project divided by the life of the project and the original investment. The limitation of this indicator is that it does not take into account the time value of money.

Integral to cost evaluation is the ability to optimise process costs. The following section presents a study of crossflow filtration, which will be the target of cost reduction in Part II of this thesis.

1.6 Crossflow filtration

Crossflow filtration or tangential flow filtration is a potentially good system for solids separation within a disposables-based process topology. In fact, microfiltration (MF) and ultrafiltration (UF) membranes can be made of polymeric substances and disposed of after each use instead of being submitted to long and costly cleaning-in-place (CIP) and sterilisation. This technique is also competitive with centrifugation in conventional bioprocessing for several reasons, as indicated in section 1.6.8. Crossflow filtration has however some limitations which include concentration polarisation and fouling, which generate low filtration fluxes. The second part of this thesis will focus on methods to overcome these limitations.

1.6.1 Background

Microfiltration and ultrafiltration are pressure-driven membrane separation processes that can be used to concentrate or purify. The feed stream is constantly circulated across the membrane surface, thereby providing a sweeping action which helps keep the membrane pores from plugging (Bailey et al., 1990). The filtrate or permeate passes through the membrane (Figure 1.1). The fluid that doesn't permeate is called concentrate or retentate.

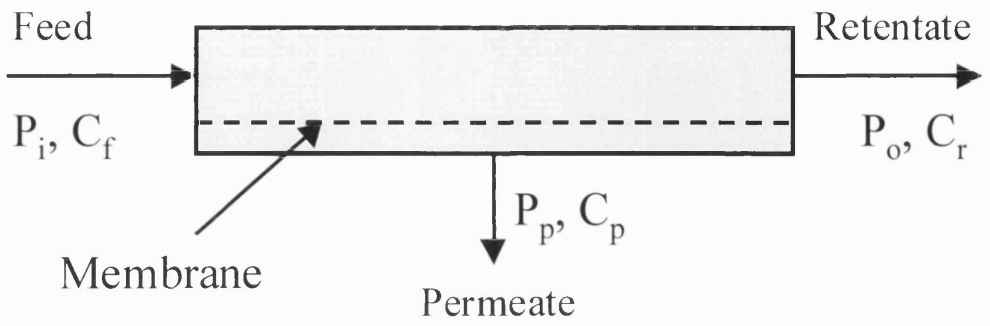


Figure 1.1 Crossflow filtration variables.

The transmembrane pressure (TMP) is the driving force in such processes and is defined by:

$$TMP = \left(\frac{P_i + P_o}{2} \right) - P_p$$

Equation 1.3

where P_i is the inlet pressure, P_o is the outlet pressure and P_p is the permeate side pressure (see Figure 1.1).

The flux (J) through the membrane may be expressed by Darcy's law, where the membrane and the cake layer (including concentration polarisation and fouling, see sections 1.6.3 and 1.6.4 respectively) are considered as two resistances in series, R_m and R_c respectively:

$$J = \frac{TMP}{\eta(R_m + R_c)}$$

Equation 1.4

where η is the dynamic viscosity of the suspending fluid (Kluge et al., 1999).

The membrane may be defined as a semi-permeable barrier between two homogeneous phases and its selectivity can be expressed by the retention factor (R):

$$R = \frac{C_f - C_p}{Cf}$$

Equation 1.5

where C_f is the solute concentration in the feed and C_p is the solute concentration in the permeate (Figure 1.1). The value of R varies between 0 (solute and solvent pass through the membrane freely) and 1 (complete retention of the solute) (Mulder, 1996). Alternatively the concept of transmission (T) can be used, defined as

$$T = 1 - R = \frac{C_p}{C_f}$$

Equation 1.6

In practice the flux increases with increasing imposed TMP until it reaches a maximum pressure-independent value called limiting flux, as a result of concentration polarisation, as will be described below in section 1.6.3.

1.6.2 Effect of pH and ionic strength

The separation achieved by MF or UF is mainly size-based but it is also affected by chemical and physical interactions between materials, the membrane and the solvent (Le and Atkinson, 1985). The ionic strength and the pH of the buffers used are especially important. For example Menon and Zydney (1999) mention cases where the transmission of bovine serum albumin (BSA) decreases by nearly two orders of magnitude as the NaCl concentration is reduced from 150 to 1.5 mM. Le and Atkinson (1985) also report maximum protein transmission with higher buffer ionic strength in lysate microfiltration. These authors interpret the low transmission at low ionic strength to be a result of an enlargement of the enzyme through swelling or association with other proteins. A similar effect is observed as a result of the pH of the buffer, with reported maximum transmission near the iso-electric point of the protein (Menon and Zydney, 1999; Le and Atkinson, 1985). This effect is also attributed to swelling. On the other hand Huisman et al. (2000) observed a minimum for BSA transmission at the iso-electric point. This was attributed to the high fouling (see section 1.6.4) levels resulting from increased hydrophobic interactions and aggregation.

Effects of ionic strength and pH are therefore dependent on the magnitude of the electrostatic and hydrophobic interactions between the different components in the system. As a result, studies made with pure protein solutions as the feed material may not address the complexity of real industrial process streams. For example Kuberkar and Davis (1999) noted that transmission decreases when another protein species is added to the protein solution, possibly through formation of a secondary protein membrane. However protein layer formation could be prevented by addition of yeast, which probably formed a cake on the membrane surface and thereby prevented protein aggregates from approaching and fouling the membrane. Lysates are especially difficult to process due to the presence of cell debris such as cell membrane, cell wall fragments and protein precipitates with sizes from 0.1 μm down to two orders of magnitude smaller (Le and Atkinson, 1985; Bailey and Meagher, 1997).

1.6.3 Concentration polarisation

The performance of a MF or UF membrane changes with time presenting a typical flux decline: there is a sharp initial drop followed by an apparent steady state after a few hours of operation (Patel et al., 1987). The discrepancies between ideal and real behaviour are due mainly to concentration polarisation and fouling effects described below. Actual process fluxes can be less than 5% of the pure water fluxes (Mulder, 1996).

Concentration polarisation arises when proteins or other large solutes create a further resistance to the flow of permeate in addition to those of the membrane and the boundary layer. These compounds are rejected by the membrane and form gel-type layers on the membrane (Cheryan, 1986), resulting in a detrimental effect on MF and UF performance.

Concentration polarisation can be modelled with the stagnant film theory (Chen, 1998). According to this model the solutes in the feed are transported to the membrane surface by convective flow and removed by permeation through the membrane or by back diffusion into the bulk (Figure 1.2):

$$JC_b = JC_p - \mathcal{D}\frac{dC}{dy}$$

Equation 1.7

where J is the flux through the membrane, C_b and C_p are the concentrations of the solute in the bulk and in the permeate respectively, $\mathcal{D}$ is the solute diffusion coefficient and y is the coordinate in the direction perpendicular to the membrane surface. Axial diffusion and axial convention terms in the stagnant boundary layer are considered to be negligible.

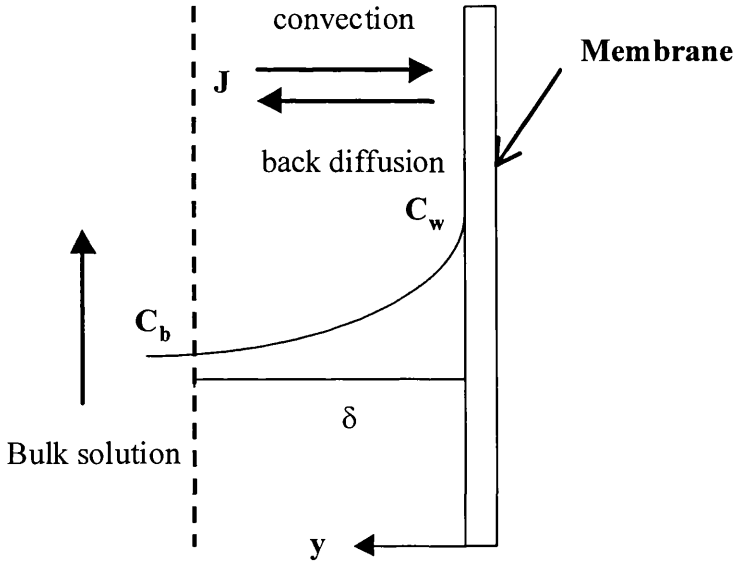


Figure 1.2 Schematic representation of the solute transport within the concentration polarisation boundary layer (adapted from Zeman and Zydney, 1996 and Le and Atkinson, 1985).

Integration over a boundary layer of thickness δ and defining C_w as the concentration at the membrane surface gives:

$$J = k \ln\left(\frac{C_w - C_p}{C_b - C_p}\right),$$

Equation 1.8

where $k = \frac{\mathcal{D}}{\delta}$ is the mass transfer coefficient.

Considering the retained solutes accumulate on the membrane surface a new concept of true transmission (as opposed to the observed transmission, defined in Equation 1.6) can be introduced:

$$T_{actual} = \frac{C_p}{C_w}$$

Equation 1.9

And:

$$T_{obs} = \frac{T_{actual}}{(1 - T_{actual}) \exp\left(\frac{-J}{k}\right) + T_{actual}}$$

Equation 1.10

The observed protein transmission is therefore expected to increase with increasing concentration polarisation as the protein concentration at the membrane surface increases (Huisman, 2000).

1.6.4 Membrane fouling

Polarisation phenomena are reversible processes, but in practice, a continuous decline in flux is observed, as well as significant changes in protein transmission, which is controlled by a combination of increasing wall concentration and solute transport (Chen, 1998). This phenomenon is called membrane fouling and may be defined as the (ir)reversible deposition of the various components present in the process stream. Fouling includes adsorption, pore blocking, precipitation and cake formation (Mulder, 1996; Zeman and Zydney, 1996). Also protein aggregation may occur due to pumping or as a result of strong electrostatic and/or hydrophobic interactions (Kelly and

Zydney, 1997). Fouling will occur as a result of the deposition of these aggregates on the membrane surface. As fouling reduces plant throughput and membrane selectivity (Marshall et al., 1997), eventually the membrane requires extensive cleaning or replacement.

An MF membrane can become fouled by components larger than its pores, which form a gel layer on the surface of the membrane resulting in decreased flux and/or transmission. Components of similar or smaller size can also cause pore plugging (Marshall et al., 1997). In particular proteins are particularly susceptible to adsorption to surfaces, and may even clog pores that are two orders of magnitude larger than the protein molecules (Le et al., 1984). According to Chen (1998) long term membrane fouling may be reduced if initial solute deposition is controlled. This can be achieved in a low fouling or polarisation regime by controlling start-up, pressure, wall concentration or flux (Chen, 1998). Constant flux operation provides better results than constant pressure operation because it avoids overfouling during initial stage of filtration (Field et al., 1995; Defrance and Jaffrin, 1999a). Effectively MF membranes have inherently high permeability values and rapid fouling will occur if the initial flux is not limited (van Reis et al., 1997b).

Field et al. (1995) considered the fouling effects on the flux to be the sum of irreversible and reversible fouling effects. According to these authors, as the TMP is increased and provided that a critical value of flux is not exceeded the behaviour is reversible, i.e. pressure can be reduced and the same fluxes are again observed. If the critical flux is exceeded, reducing the TMP doesn't restore the original flux. Operation at a constant flux just below its critical value will allow a compromise between high fluxes and long term operation without fouling (Defrance and Jaffrin, 1999b).

Shorrock and Bird (1998) defined reversible fouling as that which is "rinsable" at zero transmembrane pressure, such as loose cake and concentration polarisation. Irreversible fouling is defined as fouling that cannot be removed by rinsing and can include adhesion and pore blinding. Reversible effects associated with high concentrations at the membrane surface can lead to irreversible fouling. Cake formation should therefore be avoided by, for example, operating the membrane below the critical flux.

Other methods to minimise fouling include: pre-treatment of the feed solution (e.g., heat treatment, pH adjustment, pre-filtration); choice of adequate membrane properties (e.g., hydrophilicity, pore size); increase of the mass transfer coefficient through the use of higher crossflow velocities; promotion of turbulence to remove fouling layers and/or reduction of working pressures to avoid the compaction of the fouling layer (Mulder, 1996; Patel et al., 1987).

Crozes et al. (1997) investigated the impact of operating conditions of drinking water treatment on the irreversible fouling of UF membranes. They observed that flux recovery after a series of three backwashes was more effective when the transmembrane pressure applied during production was lower, i.e. it prevented irreversible fouling. They also concluded that concentrate velocity should be increased with increasing TMP in order to balance the forces perpendicular to the membrane surface due to the convective flux, responsible for cake formation.

Membrane performance may be improved by using turbulence or reversal of transmembrane pressure. For example the periodical removal of the transmembrane pressure by closing the permeate valve and circulating the feed solution through the membrane module can result in good flux recovery. However the performance of this method depends on the filtration period and the stopping period (Zahka and Leahy, 1985; Kuruzovich and Piergiovanni, 1996; Tanaka et al., 1995). Additionally, Tanaka et al. (1995) have further improved flux recovery under this strategy through the introduction of air bubbles into the module.

Flux recovery can also be achieved by replacing the feed stream with rinsing water. Nakanishi and Kessler (1985) investigated the effect of different variables in the efficacy of this method in the UF of skimmed milk, and included flow rate, transmembrane pressure, convective transport to the membrane due to the permeation (opened or closed permeate valve) and temperature. Their results indicate that high velocities and low TMP can achieve more than 98% removal of the deposit composed of milk proteins. Also Shorrock and Bird (1998) observed that the majority of the cellular cake can be removed with this method after the MF of yeast under strongly fouling conditions. The fouling resistance also appeared to be removed more rapidly at higher temperatures (50°C and 60°C).

Other methods make use of intermittent operation of the feed pump to increase the flux (Tanaka et al., 1995) or imposing vibrations on the filtration module (Vigo et al., 1990). One popular method is back flushing (or back pulsing), where the permeate flow direction is changed at a given frequency in order to remove the fouling layer resulting in a higher average flux (Meacle et al., 1999). This method is however not applicable to delicate membranes (e.g polymeric) that might rupture when the flow is reversed (Kuruzovich and Piergiovanni, 1996).

More recently Dean vortices devices have been exploited to reduce the extent of fouling (Kluge et al., 1999; Gehlert et al., 1998). In such modules spiral wound or helical coil design is used so that the retentate is forced to twist inducing a secondary flow with counter-rotating vortices. These re-entrain the deposits back into the bulk solution. The limitation of these devices is the additional pumping energy required, although it has been reported that their performance per unit energy is higher than that of traditional designs.

1.6.5 Membrane cleaning

Since membranes loose performance as a consequence of fouling effects they have to be regenerated between batches if they are to be reused. Cleaning methods have to be repeatable and consistent and their effectiveness is measured by comparing the pure water flux or normalised water permeability (NWP) after cleaning with the NWP before the process (Millipore, 1998a).

Chemical cleaning is considered the most important method to restore the membrane's performance (Mulder, 1996) but it is time consuming and costly as it typically requires multiple steps including (Rudolph and MacDonald, 1994): system flushing of process material with buffer, system cleaning with recirculating base, system flushing of base with reverse osmosis (RO) water, system cleaning with recirculating acid, system flushing of acid with RO water, testing efficacy of cleaning by checking NWP. For this reason the downtime associated with cleaning can account for a significant percentage (≈30%) of total cycle time (see Table 1.3). Additionally the cleaning procedure needs to be validated, which is also cost and time consuming. For this reason membranes are not always reused (Meacle et al., 1999).

Operation	Duration (hrs)	Operation Type
Test Integrity	0.25	
Concentrate Protein	4.0	Process
Pump Out Protein	0.25	**4.5 hrs (69%)**
Clean System with 0.1N NaOH	1.0	
Flush Cleaning Solution	0.25	Cleaning
Sanitize System with 50 ppm NaOCl	0.5	**2.0 hrs (31%)**
Test Integrity	0.25	
TOTAL CYCLE TIME	**6.5 hrs**	**6.5 hrs**

Table 1.3 Typical operating cycle for batch concentration (adapted from Millipore Technical Brief, 1992).

The choice of the membranes may also be conditioned by their susceptibility to cleaning. For example Bailey and Meagher (2000) found in a comparison of different membranes that the best performing membrane (cellulose acetate) could not be chosen due to difficult cleaning as a result of the sensitive nature of the polymer. This would clearly not be a problem in a process where membranes are not reused.

1.6.6 Modules and modes of operation

Crossflow filtration membranes can be found in different module configurations as shown in Table 1.4. Different modules are appropriate for specific applications and have different hold-up volumes which may affect the overall yield. One key difference between designs is the price. For example kidney dialysis cartridges (hollow fibre) can cost up to 85 fold less than traditional flat sheet (plate and frame) membranes (see Chapter 4, section 4.4.2).

	tubular	**plate-and-frame**	**spiral-wound**	**capillary**	**hollow fibre**
packing density	low →				very high
investment	high →				low
fouling tendency	low →				very high
cleaning	good →				poor
membrane replacement	yes/no	yes	no	no	no

Table 1.4 Qualitative comparison of various membrane configurations (Mulder, 1996).

A typical batch crossflow filtration system comprises a tank connected to a recirculation loop, which includes a pump and the membrane, as well as some instrumentation such as pressure gauges, flowmeters and temperature probes. In flux control mode a second pump is installed on the permeate line (see Figure 5.1 in Chapter 5).

In the case of removal of solid contaminants (e.g. clarification of cell lysates), the solution is recirculated via the loop and the purified stream passes out through the membrane. Further recovery of the product can be achieved with constant volume diafiltration, which is the addition of pure buffer to the tank at the same rate as the permeation rate. This has however the disadvantage of diluting the purified stream.

Concentration of products (e. g. proteins or cell harvesting) is achieved in the same way but in this case the membrane retains the desired product. The diafiltration step is used to wash away any contaminants (Quirk and Woodrow, 1983) and can also be used for buffer exchange, without altering the product concentration.

Another type of system is the high performance tangential flow filtration (HPTFF) system, which has been developed to reduce the pressure drop along the membrane which can be as high as 0.5 $bar.m^{-1}$ (Huisman et al., 1997). This system has been developed to facilitate a crossflow both in the feed side and on the permeate side, thus guaranteeing a uniform low transmembrane pressure. HPTFF has been reported to facilitate the separation of species of similar size (van Reis et al., 1997a).

1.6.7 Microfiltration and ultrafiltration

The pore sizes of MF membranes range from 0.05 to 10 μm, which makes it useful for the retention and concentration of micro-organisms, cellular fragments, fine precipitates, etc (Mateus et al., 1993). Applications of MF include therefore cell harvesting, cell debris removal, waste-water treatment and non-thermal sterilisation (Mulder, 1996). These applications include even the processing of more fragile cells, such as mammalian cells, since the loss of viability is not significant when a peristaltic (Ng and Obegi, 1990) or a rotary lobe pump (Rudolph and MacDonald, 1994) is used.

The pore sizes of the membranes used in UF range from 1 nm to 0.05 μm, which corresponds to 1 to 1000 kD size retention (Millipore, 1998a). It is typically used to

separate, purify and concentrate large molecules such as proteins in solution, polysaccharides, antibiotics and pyrogens. It can also be used to process cells and colloidal suspensions (Mateus et al., 1993).

Membranes with pore sizes in the range 300-1000kD are at the boundary between MF and UF membranes and can present better performance than MF for lysate clarification. In fact, since pore plugging is effected by components of similar or smaller size than the pores, it will be less prone to occur when operating with tighter membranes (Marshall et al., 1997).

1.6.8 Comparison with other separation methods

Traditionally, separations in the biotechnology industry were achieved through centrifugation, rotary vacuum filtration, filter presses and precipitation (Bailey et al., 1990; Tutunjian, 1984; Gatenholm et al., 1988). Since the 1970s however crossflow filtration has emerged as an important tool for cell harvesting and protein purification.

Crossflow filtration is competitive with centrifugation in conventional bioprocessing due to its relatively low running and capital costs, modular construction, easy scale up, higher product purity and operation at ambient temperature and in a sterile and contained environment (Shorrock and Bird, 1998; Mulder, 1996). Many centrifuges have the potential to generate aerosols and heat, have high maintenance costs and are a source of noise pollution (Stratton and Meagher, 1994). Also, the development of recombinant organisms calls for the need of greater containment (Bailey et al., 1990). Additionally, membrane technology has very low labour requirement (Gatenholm et al., 1988).

Another advantage of crossflow filtration is that it offers the versatile tool of diafiltration for buffer exchange and cell washing (Stratton and Meagher, 1994), while in the case of centrifugation, cells have to be washed by repeated centrifugation and redilution steps (Tutunjian, 1984). Also, since the membrane physically retains the cells, recovery is essentially 100% with crossflow filtration (Tutunjian, 1984).

In a disposables-based plant crossflow filtration offers the additional advantage of potential disposability, which is not an alternative for conventional centrifugation.

1.6.9 Crossflow filtration and disposables technology

In disposables-based technology the cost of membranes can become a significant term among running costs, as will be shown in Chapter 4 (section 4.3.2). This is due to the fact that they have to be disposed of after each batch together with a high cost per unit area (approx. £1200 per m^2 for flat sheet membranes, Millipore catalogue, 1999). Stratton and Meagher (1994) reckon the cost to replace membranes can result in crossflow filtration being cost prohibitive. On the other hand there are already examples of companies that do not reuse membranes (Meacle et al., 1999).

It will therefore be very important to work at achieving maximum levels of performance and hence minimum membrane areas in order to render the disposables option economically attractive. This might mean following the same approach as that of Russotti et al. (1995) taking advantage of the high initial flux rather than relying on prolonged filtration at low pseudo-steady state flux. These authors state that high initial fluxes allowed them to collect 35% of the total permeate within the first 10 minutes of the separation, while the rest of the permeate was recovered in an additional 80 minutes.

Membrane area minimization has the additional advantage of reducing the hold-up volume of the system thus increasing the achievable yields (van Reis et al., 1997b). However membrane area reduction will have to be contemplated carefully when it results in increased filtration times since there might be loss of enzyme activity (Parnham and Davis, 1995). Russotti et al (1995) considered that early downstream processing should be performed within 2 to 4 hours to avoid protease degradation. So attempting to reduce the required membrane area by opting for prolonged filtration periods does not always constitute a solution.

The disposal of the membranes has several advantages such as reduced downtime between batches due to the absence of CIP, as seen in section 1.6.5, and consequently no need for cleaning validation. It also incurs a significant saving in running costs since CIP alone can cost up to 10000 $/batch (Christy, C., 1998b). Maximum performance in the beginning of each batch can be assured by the use of new membranes.

The cost of membranes can also be expected to decline once a significant market for disposables-based technology will have been created, where the scale of membranes production will be higher and therefore compensate membrane manufacturers for the lower selling prices. New membrane fabrication techniques, such as thermoplastic injection moulding of devices (Christy, 1998a) where the production is automated will also open a door towards membrane cost reduction.

Disposable membranes will have to be self-contained, i.e. not need a holder to provide the necessary mechanical resistance, so that all wetted parts of the system are disposable. This is not the case for many types of modules, e.g. flat sheet, although membrane companies are starting to make an effort in that direction (Pellicon XL from Millipore; Sartocon Slice Disposable from Sartorius, etc).

Thanks to its versatility crossflow filtration can be used at a number of different points within the disposable bioprocess. These steps can include cell harvesting, cell debris removal, protein purification, buffer exchange for chromatography, etc.

To place disposable microfiltration in a process context it was decided to base the study upon a relevant feed stream. The next section provides details of the experimental system adopted.

1.7 Experimental system

The case study in Chapter 4 used for the economic comparison of conventional vs. disposables-based technology is the production of a periplasmic Fab' antibody fragment with *Escherichia coli*. This fragment differs from the Fab fragment by the addition of a few hinge region residues to the heavy chain C_H1 domain (Carter et al., 1992). The system is intended to be representative of biopharmaceutical processes.

Antibodies are Y-shaped tetramers of polypeptides. These antigen-specific immunoglobulins have affinity for and bind to antigens. To achieve this end, antibody molecules are built up from discrete units of genes encoding for variable segments and constant segments in a four-chain structure - two heavy chains (H) and two light chains (L). The variable segments (V_L or V_H) differ markedly from one antibody to another and are responsible for the differences in antigen binding, while the constant segments (C_H or C_L) determine the basic antibody structure. The Fab region comprises the

variable Fv region (V_L + V_H) and part of the constant region (Clark, 1995; Searle et al., 1995).

Disposable membrane filtration was illustrated with the lysate clarification step of the process indicated above. This unit operation was preferred to cell harvest or protein ultrafiltration since its increased difficulty makes membrane area optimisation more crucial. The key objective is the maximisation of antibody fragment transmission through the membrane as a function of time.

1.8 Aims of research

There is today a strong need for innovative manufacturing methods that will address at least some of the difficulties that hamper the development and commercialisation of biopharmaceutical drugs. The use of disposable equipment is seen as a possible alternative to traditional methods of processing using capital-intensive fixed plants. Due to its unique features the economic assessment of the use of disposables is not straightforward, especially considering that there is a general lack of adequate costing models for biopharmaceutical facilities.

This project aims to:

- Develop and validate suitable economic models for both conventional and disposables biopharmaceutical facilities.

- Evaluate the economic feasibility of fully disposable biopharmaceutical plants and to compare it to traditional technologies.

- Improve the economic attractiveness of disposables technology by reducing running costs. This is achieved through the development of a theoretical and experimental method to reduce membrane filtration areas.

In achieving these aims information was obtained from a panel of industrial partners, which included Mr. David Doyle (Kvaerner Process), Professor John Birch and Mr. David Sherwood (Lonza Biologics) and Mr. Charles Christy (Millipore).

1.9 Structure of the thesis

Although the contents of this thesis are divided into two distinct parts, these are strongly interrelated.

In Part I disposables-based technology is evaluated from an economic point of view and the cost of membranes is identified as a crucial factor in the economic viability of such plants. Within Part I Chapter 2 presents a review and development of models available and models are chosen for the costing of bioprocessing plants. Chapter 3 proceeds to adapt the conventional models and proposes a methodology to cost disposables-based plants. An application of this methodology to a particular case study follows (Chapter 4) together with a detailed sensitivity analysis. The comparison was made on the basis of the net present value (*NPV*), which requires the calculation of the capital investment and of the annual running costs.

Part II focuses on the optimisation of disposables-based membrane separations, which ultimately results in economic benefits for disposables-based technology. Chapter 5 details the materials and methods for the second part of the thesis. The experimental results of the microfiltration of an *E. coli* lysate are presented in Chapter 6. Chapter 7 presents the results of modelling of membrane area reduction strategies, which are then exploited experimentally in Chapter 8.

Finally the main conclusions of Part I and Part II of the thesis are presented in Chapter 9, followed by recommendations of future work in Chapter 10.

PART I

ECONOMIC ASPECTS OF DISPOSABLES-BASED BIOPROCESSING

Chapter 2 Economic models for Biochemical Engineering

2.1 Introduction

The economic viability of a project can be assessed from the calculation of the Net Present Value (NPV), which has to be positive in order for the project to be acceptable in economic grounds. This economic indicator relates the initial capital outlay required to the net profit that can be realised, the latter being the difference of product selling price and running costs. Models are required for the estimation of the capital investment and running costs.

Whereas chemical engineering is a mature subject, with many dedicated textbooks that look at the economic evaluation of plants (Peters and Timmerhaus, 1991: Holland et al. 1984 in Perry and Green, 1984; Sinnott, 1991 in Coulson and Richardson, 1991), the same does not apply to the biopharmaceutical field. Bioprocessing is a relatively recent area, still in rapid growth and with a high rate of innovation (Schmidt, 1996). The few books that have been published in the subject that look at the economic evaluation of bioprocessing plants have relied mainly on the available chemical engineering methods (Atkinson and Mavituna, 1991; Bailey and Ollis, 1986; Reisman, 1988). Papers published on economic evaluation of bioprocesses also rely on these models (Mathys et al., 1999) or modified versions of these (Farid et al., 2000a), as do modelling tools such as BioPro Designer® from Intelligen Inc. (Petrides et al., 1995). Chemical engineering models provide a good starting point for the costing of bioprocesses but they do not take into account features specific to biopharmaceutical plants such as asepsis and guaranteed containment of potentially harmful products (Datar and Rosen, 1990), validation, need for water for injection (WFI), high quality surface finishes, controlled flow of personnel and materials, etc, all of which have a large impact on costs. Papers with economic data based on personal experience (Datar and Rosen, 1990; Datar et al., 1993) or based on actual projects (Nizel and Schoenfeld, 1996; Beck, 2000; Ward, 2000) are scarce and often do not provide much detail. In fact it is likely that most companies have their own in-house costing methods, but these are unavailable to the public.

This chapter will compile the information mentioned above and with it develop a method for the economic evaluation of biochemical engineering plants, particularly those dedicated to the production of biopharmaceuticals. The first section (2.2) presents layout data for biopharmaceutical plants, broken down in terms of function area and respective costs. Section 2.3 will assess different ways of estimating the capital investment of biopharmaceutical plants, and an appropriate model will be selected. The same approach will then be taken on section 2.4 for the evaluation of the running costs. Finally the conclusions on the validity of the methods chosen will be assessed in section 2.5.

2.2 Plant Layout

Biopharmaceutical facilities are very different from chemical process plants and have to comply with current Good Manufacturing Practices (cGMPs). Separate well-defined areas must be provided for different stages of the process to prevent cross-contamination (Johnson and Stutzman, 1994), so for example personnel, equipment and materials are required to pass through air locks to access and exit controlled clean areas.

The aims of this section are to identify the different areas of containment of biopharmaceutical plants and to present a breakdown of these areas in terms of footprint and relative costs. Although this is not required at a preliminary estimate level it can be of use for more detailed estimates. It will also be of use for the subsequent economic evaluation of disposables-based plants (Chapter 3).

The plans of two different plants were used as the calculation basis. The first one was the first floor plan of a contract manufacture facility (Lonza Biologics) in Portsmouth, NH (Lonza Biologics, Biopharmaceutical Manufacturing Services brochure). The second floor plan corresponded to a pilot scale research facility (The Advanced Centre for Biochemical Engineering (ACBE) at University College London (UCL) - Phase 1).

The total area of each facility can be broken down into different areas that can be classified in terms of clean room category and according to their cost (Table 2.1). The cost information was taken from Rogers (1993). The utilities area was not provided in the floor plan of the Lonza Biologics plant but according to expert advice (Sinclair,

1999) this area can be estimated to take up 30 % of the whole facility excluding offices. This estimate is consistent with the abridged building plan figured in a CD Rom of the same contract manufacturer (Algroup Lonza, 1999).

Classification	Examples	Facility Costs ($/sq ft)*
Class 100,000	Fermentation Purification Media / Buffer Prep Wash up Autoclave	125-175
Utilities		40-70
Unclassified	Store areas Quality control labs Cell banking / storage Packaging Locker rooms Offices	60-100

Table 2.1 Description of classified areas and corresponding costs for biopharmaceutical/biotech facilities (Rogers, 1993). Facility costs include civil, structural, architectural, lighting and HVAC.

The first column in Table 2.2 shows the breakdown in terms of area cost obtained from the floor plans of the two facilities. The cost breakdown in the second column was obtained with the use of the middle value of the cost ranges shown on Table 2.1. It can be seen that these two plans give different breakdowns, which is not surprising considering that the function of both plants is very different: contract manufacturing versus research. The main disparities lie in the size of the utilities area, which is much larger in the case of the manufacturing facility. The value of 25% of the total area for utilities indicated for the contract manufacture facility is probably more adequate as it results from two different estimates.

	Area Breakdown	Cost Breakdown
Process Area (Class 100,000)	30 % / 25 %	44 % / 38 %
Media / Buffer Prep + Wash Area	5 % / 5 %	8 % / 5 %
Utilities Area	25 % / 9 %	14 % / 5 %
QC/QA Labs Area	9 % / 10 %	8 % / 8 %
Storage Area	5 % / 3 %	4 % / 2 %
Other	26 % / 48 %	22 % / 42 %
Total	**100 %**	**100 %**

Table 2.2 Area and Cost Breakdown for two bioprocessing plants (Lonza Portsmouth, NH / ACBE, UCL).

2.3 Capital Investment

2.3.1 Summary

There are several methods with which to estimate the initial investment required to build a new biopharmaceutical plant. These differ in the level of accuracy and of detail that they offer.

In a simple method the capital investment can be estimated from the size of the main fermenter (Jacobs Engineering company publication, 1997):

$$\text{Capital Cost } (£\times 10^6) = 0.0017 \times \text{Fermenter Capacity (L)} + 10.79$$

Equation 2.1

Although this approach provides a quick rule of thumb to estimate overall capital investment costs it provides no knowledge of the individual costs that constitute it.

In another approach initially proposed by H. J. Lang for chemical engineering plants (Lang, 1947a; Lang, 1947b; Lang, 1948; Peters and Timmerhaus, 1991), the fixed capital investment (FCI) can be calculated by multiplying the equipment cost by a factor, which depends on the type of process plant being used. The specific value for such a factor applicable to bioprocessing plants is not easily available from the literature but can be obtained from the sum of the individual factors that constitute the fixed capital investment.

The fixed capital investment for a bioprocessing plant (FCI_{conv}) is therefore given by:

$$FCI_{conv} = L_{conv} E_{conv} = c(\sum_i f_i) E_{conv}$$

Equation 2.2

where L_{conv} is a "Lang" factor for bioprocessing plants and E_{conv} is the cost of the process and utilities equipment. The factors f_i relate to E_{conv} to give the cost of items such as pipework and installation, process control, instrumentation, electrical power, building, detail engineering, construction and site management, commissioning and validation. A contingency factor, c, may also be included. The index $_{conv}$ used throughout this thesis refers to bioprocessing plants based on conventional stainless steel equipment so as to make a distinction from disposables-based plants, which will be analysed later in Chapter 3.

The sum of the fixed capital investment with the working capital (additional investment needed to start the plant up and recovered at the end of the project) constitutes the total capital investment (Sinnott, 1991).

For fluid processing chemical engineering plants a value of 4.83 is suggested for the Lang factor, or 5.1 including building construction (Peters and Timmerhaus, 1991). According to Lang (1947b) this method provides an accuracy of 10%. Typical Lang factors for pharmaceutical and fine chemicals plants are in the range of 6 to 8 (Osborne, 1997), which will be considered here a good approach to biopharmaceutical plants.

2.3.2 Breakdown based on chemical engineering plants

This method is based on data for fluid-processing chemical engineering plants and the individual items and factors to be used with Equation 2.2 are shown in Table 2.3 (Peters and Timmerhaus, 1991).

ITEM	f_i
Direct Costs	
Purchased Equipment - Delivered	**1**
Purchased Equipment - Installation	0.47
Instrumentation and Controls - Installed	0.18
Piping - Installed	0.66
Electrical - Installed	0.11
Buildings - Including Services	0.45
Yard Improvements	0.10
Service Facilities - Installed	0.70
Land (if purchase is required)	0.06
Indirect Costs	
Engineering and Supervision	0.33
Construction Expenses	0.41
Contractor's Fee	0.21
Contingency factor (c)	1.1
LANG FACTOR	**5.1**

Table 2.3 Factors for estimating capital investment items based on the cost of the delivered equipment (adapted from Peters and Timmerhaus, 1991). Note: the building costs correspond to a new plant at a new site (grass roots).

The overall Lang factor at 5.1 is less than the lower limit of the range suggested above. In fact this factor excludes costs that are specific to biopharmaceutical plants; for example validation costs. The building costs were established for chemical engineering plants that do not require HVAC, segregated areas, special seals and surface finishes, etc. This factor should therefore be higher than 0.45 for bioprocessing plants. The cost of service facilities is also likely to be higher in biopharmaceutical plants, which in addition to traditional utilities require WFI, a kill tank, etc. The cost of instrumentation and controls may also increase for bioprocesses due to the specific conditions required for temperature, oxygen and mixing (Datar and Rosen, 1990).

Petrides et al. (1995) used a similar breakdown in the development of the simulation tool BioPro Designer®, Intelligen, Inc. (Table 2.4). The main difference is that indirect costs are much higher than in the example above, although still within the ranges suggested by chemical engineering textbooks (Peters and Timmerhaus, 1991). Building and instrumentation costs are also higher than in the previous breakdown,

which should be a better approximation to a bioprocessing plant. The resultant Lang factor is therefore closer to what would be expected for biopharmaceutical plants but this model still excludes validation costs. Also the cost of facilities was reduced in this model, which is contradictory to what is expected from bioprocesses. Despite these facts BioPro Designer® and SuperPro Designer®, both from Intelligen, Inc. (Scotch Plains, NJ, USA), have been widely used in economic studies of biopharmaceutical plants (Petrides et al., 1995; Ernst et al., 1997; Erickson, 1993) as well as non-biopharmaceutical (Choi and Lee, 1997).

ITEM	f_i
Direct Costs (DC)	
Purchased Equipment	**1**
Installation	0.51
Instrumentation	0.50
Process Piping	0.35
Electrical	0.10
Buildings	0.85
Yard Improvement	0.15
Auxiliary Facilities	0.60
Insulation	0.03
Indirect Costs (IC)	
Engineering (25% of direct costs)	1.02
Construction (35% of direct costs)	1.43
Contractor's Fee (5% of DC+IC)	0.33
Contingency (10% of DC+IC)	0.65
LANG FACTOR	**7.52**

Table 2.4 Factors for estimating capital investment items based on the cost of the delivered equipment (adapted from Petrides et al., 1995).

2.3.3 Breakdown based on biochemical engineering plant data

The fixed capital investment factors presented in Table 2.5 are based on bioprocessing plant project data (Sinclair, 1999). Following the advice of the industrial partners involved in this project, some adjustments were made to the initial data so as to

increase the contribution made by validation costs. According to Doyle (2000) the costs of validation can be divided into two elements:

- 2 to 5% of the overall costs for the validation master plan and installation qualification (IQ) and performance qualification (PQ) protocol preparation and execution.

- 15 to 20% of the overall costs for the preparation of the standard operating procedures (SOPs), staff training, process qualification and process validation.

An average of 15% of the fixed capital investment was therefore taken for validation costs. This value is also the middle of the range indicated by Van Horn (1999). This takes the original validation factor of 0.18 to 1.06, as shown in Table 2.5.

i	Description	f_i
1	Equipment and utilities	1
2	Pipework and installation	0.9
3	Process control	0.37
4	Instrumentation	0.6
5	Electrical power	0.24
6	Building works	1.66
7	Detail Engineering	0.77
8	Construction and site management	0.4
9	Commissioning	0.07
10	Validation	1.06
	Contingency factor (c)	1.15
	"Lang" Factor	$L_{conv} = 8.13$

Table 2.5 Capital investment factors (f_i) for a conventional bioprocessing plant and corresponding "Lang" factor (Novais et al., 2001).

The factors f_1 to f_{10} in Table 2.5 can be used in Equation 2.2 and enable the calculation of the "Lang" factor for conventional bioprocessing plants, L_{conv} (Novais et al., 2001). This factor, evaluated at 8.1 is again close to the range quoted for this type of plant (6 to 8). It has to be noted however that in this breakdown the costs of utilities are included with those of process equipment. This is not the case in the

original Lang method and in the methods presented in section 2.3.2. The consequence is that the Lang factor obtained here is not directly comparable with those for chemical engineering. If the utilities costs had been considered as a separate term the Lang factor would be higher than 8.1, depending on the weight utilities take in the total equipment costs.

2.3.4 Other biochemical engineering breakdowns

Osborne (1998) suggests different factors and items to the ones above in order to establish the total capital costs of a pharmaceutical pilot plant, as shown in Table 2.6. It can be seen that the total Lang factor for this estimate (L=6.37) is within the normally accepted range above but does not take into account a contingency factor, which would bring the Lang factor up to approximately 7.

ITEM	f_i
(Equipment (delivered)	1)
Services (EPCm)	1.8
Pipework	0.2
Mechanical erection	1.15
Electrical Instr. materials and erection	0.88
Building materials and erection	1.76
Control System	0.21
Site Facilities	0.37
LANG FACTOR	**6.37**

Table 2.6 Factors for estimating capital investment items based on the cost of the delivered equipment (adapted from Osborne, 1998). EPCm: Engineering Procurement and Construction Management.

The capital investment can also be calculated according to a method proposed by Doyle (2000). The costs associated with the process, which include piping, valves, automation, installation, etc, can be obtained according to:

Total Process Plant Cost = Total equipment cost × Installation factor

Equation 2.3

In this equation the total equipment costs also include those of the utilities and the installation factor is said to be typically 2.2 to 3.0 for biotech facilities. Taking the middle value of the range suggested by this author (2.6) and assuming process costs constitute 40% of the capital costs (the remaining 60% of the costs being associated with the buildings, validation, etc), one can estimate the Lang factor at 6.5. With the inclusion of a 10% contingency factor this estimate becomes 7.15, which is again compatible with the range suggested previously.

In their paper Nizel and Schoenfeld (1996) presented the costs for the construction of Genzyme's Cerezyme production plant. Of a total $100 million project cost, process and piping costs accounted for approximately one third. Considering that process costs can be obtained from the equipment costs multiplied by a factor of 2.2-3 (Doyle, 2000), an overall Lang factor can be calculated at approximately 7.5. This value obtained from real data validates the range of 6 to 8 suggested above.

There also exist multiple-factor methods, not mentioned here due to their difficult use, which divide the capital investment into two cost categories: labour related costs and material related costs. One such method was used by Datar and Rosen (1990) originating a fixed capital investment that is 7.1 times the purchased equipment cost (including utilities), once more within the range suggested for bioprocesses.

2.3.5 Chosen model

The model presented in section 2.3.3 above will be used in this thesis as the preferred model for costing the capital investment of biopharmaceutical plants for the following reasons:

- It is based on real biopharmaceutical plant data.

- It provides detailed information on the different cost items that constitute the capital investment. Consequently it also constitutes a good starting point for a model to cost disposables-based plants.

- The overall Lang factor resulting from this breakdown is close to the range suggested by Osborne (1998). It is also consistent with other models presented. For

example the installation factor of 2.87 is within the range indicated by Doyle (2000) (section 2.3.4).

- It has been discussed and improved by industrial experts.

2.4 Running costs

2.4.1 Summary

As in chemical engineering, the operating costs of bioprocessing plants can be divided into variable costs (e.g. materials, direct operating labour) and fixed costs (e.g. maintenance). Most models used in the estimation of biochemical engineering running costs are therefore based on those used for chemical engineering plants.

The characteristic of traditional operating cost models (section 2.4.2 below) is that they require a detailed mass balance in order to evaluate raw materials consumption, utilities and direct operating labour. Other items can then be factored in as a function of those costs, e.g. supervision costs are calculated as a function of direct operating labour costs.

The problem is that a high level of detail is not always available at a preliminary estimate stage. Additionally, biopharmaceutical processes have higher levels of variability and uncertainty, each of which result in increased complexity and production costs (Schmidt, 1996). It can therefore be useful to survey the information available on bioprocesses, in order to establish trends on the weighted breakdown of biopharmaceutical operating costs into its different components. In this analysis a distinction between bacterial (section 2.4.3) and mammalian cell processes (section 2.4.4) is necessary, as these two types of processes present fundamental differences.

2.4.2 Chemical engineering model

The first model used to evaluate running costs was originally developed for traditional chemical engineering processes (Sinnott, 1991). The running costs (*RC*) are composed of direct production costs (*DPC*), which include fixed costs (*FC*) and variable costs (*VC*) and general operating expenses (*GOE*), which include R&D costs, sales expenses, etc. For simplification purposes the general operating expenses will not be

considered in this analysis. These costs have also been excluded in other studies (Petrides, et al., 1995; Farid et al., 2000a). Hence:

$$RC = DPC + GOE = FC + VC + GOE \approx FC + VC$$

Equation 2.4

In this model the fixed costs are estimated as percentages of the operating labour costs and of the capital investment according to the following equation, adapted from Sinnott (1991):

$$FC_{conv} = OL_{conv} \sum_{i=1}^{4} g_i + FCI_{conv} \sum_{i=1}^{4} h_i$$

Equation 2.5

where FC_{conv} and OL_{conv} are the fixed running costs and the operating labour costs in the conventional plant respectively, g_1 to g_4 are factors which multiplied by OL give the cost of operating labour ($g_1 = 1$), supervision, Quality Control and Quality Assurance (QC/QA), and plant overheads respectively, FCI_{conv} is the fixed capital investment of the conventional plant and h_1 to h_4 are factors which multiplied by FCI give the cost of tax, insurance, maintenance and depreciation respectively. Operating labour (OL) includes all hands-on process plant in single shift plus skeleton staff out of hours. Supervision includes all direct line managers for production. QC/QA corresponds to all persons involved in Quality Control and Quality Assurance and plant overheads include general management, plant security, general clerical staff, safety, etc (Sinnott, 1991).

The differences between traditional chemical engineering and biopharmaceutical plants are again visible when looking at the running costs. For example the quality staff of a biologicals contract manufacturer (Lonza Biologics) include a validation team, a compliance group, a documentation team, a regulatory team and the Labs staff (Sherwood, 2001). Only the latter would be needed in a traditional chemical engineering plant. The cost estimate of QC/QA (denominated Laboratory costs in the original model) was therefore increased here from 20 to 40% of the operating labour.

This value is also consistent with the staff costs breakdown of Lonza Biologics (Pugh, 1998).

Table 2.7 shows the values g_1 to g_4 and h_1 to h_4 to be used with Equation 2.5. The difficulty is to evaluate the operating labour (OL) costs adequately, as this requires a high level of understanding of the plant that is being assessed.

i	Description	model	g_i	h_i
1	Operating Labour	*OL*	1	-
2	Supervision	20% *OL*	0.2	-
3	QC / QA	40% *OL*	0.4	-
4	Plant Overheads	50% *OL*	0.5	-
1	Tax	2% *FCI*	-	0.02
2	Insurance	1% *FCI*	-	0.01
3	Maintenance	10% *FCI*	-	0.1
4	Depreciation	12.5% *FCI*	-	0.125

Table 2.7 Fixed running costs model based on traditional chemical engineering (adapted and modified from Sinnott, 1991) and values of g_i and h_i factors. The factors g_i and h_i are defined as in Equation 2.5.

In the same way, the variable costs have to be estimated based on the process requirements and include the cost of the raw materials, of consumables such as membranes and chromatography matrices and of utilities. Again, the evaluation of these costs requires detailed knowledge about the facility, which can be difficult at initial stages. A rough estimate of the utilities running cost can be obtained considering that a bioprocessing facility has a utility bill of approximately £200 per year per m^2 of manufacturing area (Sawyer, 1999). This is dependent upon an estimate for the footprint of the facility being available. Farid et al. (2000a) also used the model in Table 2.7 but with no modifications (QC/QA costs were taken as 20% of operating labour costs as in Sinnott, 1991).

A similar model is used in the simulation tool BioPro Designer®, Intelligen, Inc (Petrides et al., 1995), shown in Table 2.8. The main differences lie in the fact that this

tool makes a separate estimate of the maintenance costs, divided into maintenance labour costs and maintenance materials costs and utilises the former for the calculation of supervision costs. This model also considers additional items such as fringe benefits and operating supplies. The estimate of laboratory costs is 30% of operating labour, and somewhat higher than the traditional chemical engineering estimates. In this breakdown plant overheads are calculated as a function of the sum of operating labour, maintenance labour and fringe benefits and its estimate is also significantly higher than in the chemical engineering model.

An equation similar to Equation 2.5 can be developed from this model, where *ML* are the maintenance labour costs and *MM* are the maintenance materials costs:

$$FC = OL\sum_i g_i + ML\sum_i k_i + FCI\sum_i h_i + MM$$

Equation 2.6

The values of the factors g_i, k_i and h_i are indicated in Table 2.8.

Description	model	g_i	k_i	h_i
Operating Labour	*OL*	1	-	-
Maintenance labour	*ML*	-	1	-
Fringe benefits	*FB*=40%(*OL*+*ML*)	0.4	0.4	-
Supervision	20%(*OL*+*ML*)	0.2	0.2	-
Operating supplies	10% *OL*	0.1	-	-
Laboratory	30% *OL*	0.3	-	-
Administration and overhead expense	60%(*OL*+*ML*+*FB*)	0.84	0.84	-
Tax	2% *FCI*	-	-	0.02
Insurance	1% *FCI*	-	-	0.01
Maintenance material	*MM*	-	-	-
Depreciation	12.5% *FCI*	-	-	0.125

Table 2.8 Fixed running costs model based on BioPro Designer® operating costs model (adapted from Petrides et al., 1995) and values of g_i, h_i and k_i factors. The factors g_i, h_i and k_i are defined as in Equation 2.5.

Again the variable costs will need to be evaluated separately based on the process requirements. The running costs breakdown obtained by Petrides et al. (1995) with this model for an inclusion body *E. coli* process will be discussed below (Table 2.9 and Table 2.10, section 2.4.3).

2.4.3 Bacterial process model

A model based on a bacterial fermentation process was derived from the breakdown of the running costs observed by Datar, et al. (1993) for their particular case study. According to these authors, the annual total production cost of tissue plasminogen activator (tPA, expressed intracellularly in an insoluble denatured form) can be broken down as follows: direct manufacturing expense (47%), indirect manufacturing expense (9%), depreciation (13%) and general expense (31%). The direct manufacturing expense can be split up further into: labour (22%), fermentation materials (1%), recovery materials (8%), utilities (20%), patents/royalties (20%), waste treatment (16%) and other (13%).

The detailed breakdown was reduced down to five categories (labour, materials, utilities, depreciation and other costs) and adapted so as to exclude general expenses from the overall running costs for simplification purposes, as done in section 2.4.2. This results in (Novais et al., 2001):

$$RC_{conv} = RC_{conv} \sum_{i=1}^{5} x_i$$

Equation 2.7

where RC_{conv} is the running cost of the conventional plant, x_1 to x_5 are the fractions of the running cost which give the cost of its individual components: labour (x_1), materials (x_2), utilities (x_3), depreciation (x_4) and other costs (x_5). Other costs include patents and royalties, waste treatment and indirect manufacturing expenses such as plant overhead, tax and insurance.

The factors x_1 to x_5 obtained from Datar, et al. (1993) are presented in the first column of Table 2.9. The cost of depreciation is estimated by dividing the capital investment (FCI_{conv}) by the working life of the plant. From there it is possible to

calculate the cost of the other individual items of the running costs of the conventional plant through Equation 2.7.

Similar analyses were performed with the operating costs breakdown presented by Datar and Rosen (1990) and by Petrides et al. (1995) for inclusion body-based *E. coli* processes (second and third columns of Table 2.9 respectively).

	item	Datar et al. (1993)	Datar and Rosen (1990)	Petrides et al. (1995)
x_1	Labour costs	0.14	0.21	0.05
x_2	Materials	0.06	0.17	0.38
x_3	Utilities	0.14	0.12	0.003
x_4	Depreciation	0.19	0.11	0.24
x_5	Other	0.47	0.39	0.29

Table 2.9 Running costs factors derived from the cost distributions presented by Datar, et al. (1993), Datar and Rosen (1990) and Petrides et al. (1995) for different bacterial processes. Other costs include patents and royalties, waste treatment and indirect manufacturing expenses such as plant overhead, tax and insurance.

The different cost items in the three examples above can be rearranged into fixed, semi-variable and variable costs and their relative weights compared to those indicated by Kuhn (2000) for a bacterial process (Table 2.10). Fixed costs include depreciation, semi-variable costs include those costs indirectly tied to production such as utilities, supplies, staff support, outside expenses and variable costs include raw materials and direct labour costs. The results from Datar et al. (1993) are consistent with those suggested by Kuhn (2000) and will therefore be adopted in preference to those of Datar and Rosen (1990).

The results from Petrides et al. (1995) are also consistent with Kuhn (2000) but the cost of utilities at 0.3% of the operating costs (Table 2.9) is very low when compared to literature sources, which indicate a value in the range of 5 to 20% of the manufacturing cost (Atkinson and Mavituna, 1991).

Cost Type	Kuhn (2000)	Datar et al. (1993)	Datar and Rosen (1990)	Petrides et al. (1995)
Fixed	26%	21%	12%	24%
Semi-variable	27%	30%	30%	26%
Variable	47%	49%	58%	50%

Table 2.10 Comparison of the operating costs breakdown (exc. general expenses) from different sources: Kuhn (2000), Datar et al. (1993), Datar and Rosen (1990) and Petrides et al. (1995).

2.4.4 Mammalian cell culture model

A comparable analysis was carried out based on the running costs breakdown for a mammalian cell process (Datar et al., 1993). Although the cost items are the same and may also be calculated through Equation 2.7, their individual weights are different from those obtained from the bacterial process, as can be seen from the comparison of Table 2.9 with Table 2.11. Another breakdown for mammalian cell culture based on data presented by J. Beck (2000) is shown in Table 2.12. (Other direct costs include power and utilities, waste disposal and royalties; fixed charges correspond to depreciation of capital and taxes and insurance; plant overhead relates to supervisory labour, maintenance, QA/QC and supplies.)

i	item	x_i
1	Labour costs	0.09
2	Materials	0.38
3	Utilities	0.15
4	Depreciation	0.06
5	Other	0.32

Table 2.11 Running costs factors derived from a cost distribution presented by Datar, et al. (1993) for a mammalian cell process. Other costs include patents and royalties, waste treatment and indirect manufacturing expenses such as plant overhead, tax and insurance.

i	item	x_i
1	Operating labour	0.12
2	Raw Materials	0.26
3	Other direct costs	0.15
4	Fixed charges	0.29
5	Plant overhead	0.18

Table 2.12 Manufacturing cost breakdown for a mammalian cell process (Beck, 2000). Other direct costs include power and utilities, waste disposal and royalties; fixed charges correspond to depreciation of capital and taxes and insurance; plant overhead relates to supervisory labour, maintenance, QA/QC and supplies.

In particular it is to be noted that the relative weight of the materials costs in Table 2.11 is much higher than in the bacterial fermentation case (section 2.4.3). The same is observed with the case in Table 2.12 but to a lesser extent. Rosenberg (2000) also indicates an increase in the relative weight of raw materials from 32% in a bacterial process to 37% of the running costs in a mammalian cell culture process. In fact the cost of mammalian cell culture media is at least 5 to 10 times more expensive than media for bacterial fermentation (Willoughby, 2001).

Pugh (1998) suggests a cost distribution for the upstream part of a mammalian cell process (Table 2.13). In this case the weight of the raw materials is significantly more modest but this may not be representative of the whole process: for example Datar and Rosen report for an *E. coli* process that the total cost of fermentation media only accounts for 10% of the overall materials costs.

i	item	Range	x_i
1	Staff costs	30-55	0.42
2	Raw Materials	5-15	0.10
3	Facilities and eng.	3-7	0.05
4	Depreciation	20-30	0.25
5	Facilities overhead	15-20	0.18

Table 2.13 Manufacturing cost breakdown for the upstream side of a mammalian cell process (Pugh, 1998).

2.4.5 Chosen models

Although the chemical engineering model presented in section 2.4.2 is very comprehensive in its coverage of the different cost items that constitute the running costs, it is less dependable at an early stage of cost estimation where there is little detailed information about the process. For example the model requires a thorough knowledge of the number of operators needed to run the plant as well as their salaries. Information is also needed on the exact amounts of each raw material and their bulk price, etc. The very low estimate of utilities in the paper by Petrides et al. (1995) is a further example of where such a model can lead to incorrect results. Also, this model was developed for chemical processes, whose operating costs may split differently from those for bioprocesses.

The cost distributions presented in sections 2.4.3 and 2.4.4 are less informative and only provide a very approximate estimate of the running costs. However they do give an indication of the weighed breakdown of the different operating costs of a bioprocess. They also allow the calculation of an estimate of the overall running costs from the sole knowledge of the capital investment. These models will therefore be used in this thesis for the evaluation of the operating costs of biopharmaceutical plants, the choice of model to use being based on the nature of the process being analysed (bacterial vs. cell culture).

The breakdown obtained from Datar et al. (1993) was chosen for the evaluation of bacterial processes, as its split into fixed, variable and semi-variable costs is close to that indicated by Kuhn (2000) for a real process.

The split presented by Beck (2000) will be preferred for the evaluation of mammalian cell based process as it is also founded on real industrial data. The only problem is that this model does not indicate in detail the percentage taken by utilities costs, which is a crucial factor that will be needed later in the evaluation of disposables-based plants (Chapter 3). Utilities costs can however be estimated from the item "other direct costs", which includes utilities, waste disposal and royalties. Using the same relative weight of these 3 items relative to each other as in the mammalian cell process in Datar et al. (1993), utilities can be estimated to account for 40% of the "other direct costs" or 6% of the total operating costs.

2.5 Conclusions

The capital investment of a biopharmaceutical plant can be evaluated through the model in Equation 2.2 with the factors presented in Table 2.5. According to this model a process based on a 300 L fermentation requires a capital investment of £12.8 million (see chapter 4). This cost is not far off the value of capital investment obtained from Equation 2.1 for this volume of fermentation, which is £11.3 million.

Care must be taken when defining what constitutes the equipment costs item. There seems to be a general confusion in the literature on this matter. For example, Petrides et al. (1995) consider only process equipment when estimating equipment costs but Datar and Rosen (1990) include additionally the cost of a refrigeration unit and of the kill tank, and so provide a different analysis. The model chosen for capital investment evaluation (Table 2.5) assumes all utilities equipment costs are part of the equipment costs item.

If a more detailed evaluation of capital investment is needed building costs can be evaluated separately. The area and cost distribution obtained from the study of the Lonza contract manufacture facility provides a reliable framework for the evaluation of the layout of biopharmaceutical plants.

The evaluation of the running costs presents more difficulties. When sufficient information is available the model in section 2.4.2 can be used, bearing in mind that it was initially developed for chemical processes. For a quick rough estimate for a bacterial process it is possible to use the model developed in section 2.4.3 from Datar et al. (1993). In absolute terms, considering the same example of a 300 L fermentation, the depreciation costs will be £1.3 million per year for a 10 years plant lifetime. As this accounts for 19% of the running costs, the total value would be £6.8 million/year. Lawlis et al. (1998) indicate that the annual production costs for Phase III clinical trials of a recombinant DNA product are around $10 million, i.e. £6.7 million, very close to the previous estimate. The values obtained through this model are however very sensitive to the length of plant life chosen, since this will determine the depreciation costs.

For mammalian cell processes the breakdown suggested by Beck (2000) will be considered adequate, as it is based on a real industry case.

In summary this chapter developed a costing framework for biopharmaceutical processes. The next chapter will make use of this framework as a basis to build up similar economic models for disposables-based bioprocessing plants

Chapter 3 Economic models for disposables-based bioprocessing plants

3.1 Introduction

The economic models developed in Chapter 2 are not adequate for costing of disposables-based plants due to the unique features of this mode of operation. In such plants equipment capital costs are switched to consumables as needed and there is a reduction in maintenance as well as cleaning and steaming operations, etc. This means that on the one hand the capital investment is reduced but on the other hand operating costs may increase, depending how the rise in consumables costs is balanced out by the decrease in utilities costs, etc.

Biopharmaceutical plants have so far not been built on a fully disposable basis. The problem is that before a company takes on the challenge of building a disposable plant more information is required on how much a fully disposable plant would cost to build as compared to a conventional one. The only solution is to develop economic models specific to disposables-based plants and use these for decision-making. Afterwards the accuracy of the models can be confirmed against actual data.

This chapter will start by analysing how the layout of a disposable plant may differ from that of a conventional biopharmaceutical plant and from there infer the implications on the buildings costs (section 3.2). The subsequent sections (3.3 and 3.4) present models for the evaluation of both capital investment and running costs of disposables-based plants. These models were developed from those outlined in the previous chapter with the use of assumptions derived from characteristics specific to disposable equipment. The assumptions used were validated through sensitivity analysis in section 3.4.4 as well as through discussions with the industrial partners. Section 3.5 will present the impact of the use of disposables on time to market. Finally general conclusions will be drawn from the chapter and presented in section 3.6.

3.2 Disposables-based plant layout

The aims of this section are to predict the differences in the building area between conventional and disposables-based plants (by functional area) and from there work out an estimate for the area and hence the costs of a disposable plant.

Based on the area breakdown obtained for the conventional plant in Table 2.2 in Chapter 2 it is possible to predict how the area will change if the facility considered is based on disposable equipment.

Several assumptions had to be made, following discussions with industrial experts:

- The inoculation laboratory, the fermentation and purification areas and respective corridors constitute the Process Area (Chapter 2) and can be fused into one single area where the relevant equipment is wheeled in or out according to need. The area of this process suite will be half of the sum of the 3 previous areas.

- The storage area will be considered to double since there will be a need for additional storage place for equipment that is not being used at a particular stage of the process.

- Equipment wash and media preparation areas are not needed in the disposable plant.

- The area needed for utilities will be considered to be half of the equivalent area in the conventional plant since the utilities requirements in the disposable plant will be much lower.

- All the remaining areas will be considered unchanged.

An area and cost breakdown for the disposable facility can be arrived at from the assumptions above and from Table 2.2, as summarised in Table 3.1. The percentages are expressed in terms of the conventional plant totals.

	Assumption	Area Breakdown	Cost Breakdown
Process Area (Class 100,000)	½	15 %	22 %
Media / Buffer Prep + Wash Area	0	0 %	0 %
Utilities Area	½	12 %	7 %
QC/QA Labs Area	=	9 %	8 %
Storage Area	×2	10 %	8 %
Other	=	26 %	22 %
Total	-	72%	67%

Table 3.1 Area and cost breakdown for a disposables-based bioprocessing plant.

From this analysis it can be seen that both the area and the cost of the building for the disposable facility are reduced to approximately 70% of those for the conventional plant.

3.3 Capital Investment

3.3.1 Introduction

Intuitively it is expected that the capital investment will be strongly reduced in a disposables-based approach, due to the absence of stainless steel equipment and piping, the reduction in utilities requirements, the reduction in building area, etc. A detailed model for the capital investment will be needed in order to evaluate the overall impact of these different effects. A simple model like the one presented in equation 2.1 in chapter 2 would be of no use here because it cannot be adapted in order to accommodate the disposable features. The chosen method shown in Chapter 2 (section 2.3.3) is an adequate starting point, as it provides a detailed breakdown of the different items that contribute to the capital investment.

In this analysis the capital investment of the disposables-based plant will be calculated based on the equipment of the equivalent conventional plant, i.e. of a plant with exactly the same characteristics but based on traditional technology. This approach is taken because it is expected that disposable equipment costs and the remainder capital investment items will not correlate in the same way as in the conventional plant.

The fixed capital investment for a bioprocessing plant based on disposable equipment (FCI_{disp}) may be estimated from the cost of the installed equipment and utilities for a conventional plant as follows (Novais et al., 2001):

$$FCI_{disp} = L_{disp} E_{conv} = c'(\sum_i f_i f_i') E_{conv}$$

Equation 3.1

where L_{disp} is a "Lang" factor for disposable bioprocessing plants, E_{conv} is the cost of the process and utilities equipment as in Chapter 2 and f_i' are factors which translate the cost of the individual elements which constitute the capital investment of the conventional plant into the cost of elements for the disposable option. In other words these factors indicate how each of the capital investment items will be reduced or increased with the use of disposables. c' is the relevant contingency factor and should be identical or higher than c, depending on whether it is considered that the disposables-based plant estimates carry the same or greater levels of uncertainty.

3.3.2 Assumptions

The factors f_i' for the conversion from conventional to disposable can be estimated from the following assumptions (developed from Novais et al., 2001):

- **Equipment:** In a plant using disposable process equipment the capital investment costs for process equipment are strongly reduced. Although all equipment costs will be operating costs there are some basic items that are bought only once, such as structural items that do not have direct contact with the process streams. Examples of these include the drums that hold the bioprocessing bags and prevent them from collapsing. The cost of these fixed items sums up to less than 1% of the process equipment costs of the conventional equivalent plant (values shown in Appendix 2).

- **Utilities:** A disposables-based plant would need reduced or even no clean-in-place and steam-in-place capabilities. As a consequence the cost of utilities equipment is substantially reduced as only features such as cooling water, chilled water, process air and vacuum will be required if all of the process can be turned over to

disposable operation (Table 3.2). Opting to buy WFI in bags can replace the need for a WFI package.

Developed Utilities	Cost (£k) (Doyle, 1999)	Status in disposable plant
Compressed Air	130	Needed
WFI Package	180	Absent
Purified Water Package	160	Debatable
Clean Steam Package	140	Absent
Chilled Water	70	Needed
Glycol Water	50	Needed
Kill Tank System	100	Needed

Table 3.2 Description and costs of developed utilities (Doyle, 1999) and how they may differ in a disposable plant (Note: This table excludes main utilities such as Natural gas, plant steam, fire water, electricity, drainage and mains water, which are accounted for in the buildings costs).

- **Pipework and installation:** The capital costs associated with pipework are decreased substantially in a disposable option since the cost of the disposable tubing becomes an operating cost. Connections to utilities that do not come into contact with the product stream would normally be considered as non-disposable. Installation costs are also decreased due to the reduction in fixed equipment.

- **Process control:** Process control costs are likely to remain unchanged although in the disposable case there may well be a move toward more manual operation in the interest of speed to market. Conversely the need for more non-invasive monitoring may lead to greater costs in computing for data interpretation for control purposes.

- **Instrumentation:** Instrumentation capital costs are reduced since some of the instruments may be disposable (for example thermocouples) and therefore appear as a running cost. Alternatively instrumentation may be redesigned to be non-invasive (e.g. UV detectors) and hence lead to no change in capital cost. Other instrumentation such as gas mass spectrometers are not in contact with the process material and also do not lead to a change in capital cost, as they will be needed for

both modes of operation. Where disposable alternatives to high cost invasive instrumentation (e.g. pH meters) are not available then either separate validation for turn around (e.g. cleaning and recalibration) must be put in place or recourse is needed to data interpretation from actual available measurements (e.g. cell density by optical window, exit gas analysis, etc). Again in such a case it is assumed the capital cost is not affected.

- **Electrical power:** Assuming power consumption and capital costs are related (Lang, 1947a) it is likely that electrical power capital costs are independent of whether conventional or disposable equipment is used. Alternative methods of mixing for a disposable process are likely to have similar power requirements to those for a conventional process. Conversely a reduction in size of facility could lead to a significant decrease in air conditioning costs since this will be related to the volume of the facility.

- **Building:** The variation of the cost of the building has been estimated in section 3.2. The effect of changes in the function of the areas and consideration of their differential costs leads to a reduction in building costs when using a process based on disposable equipment.

- **Detail engineering:** The costs associated with detail engineering are expected to be reduced for the disposables option due to the less refined construction needed.

- **Construction and site management:** Construction and site management costs should be decreased due to the smaller building area required for the disposable option.

- **Commissioning:** The commissioning costs of the disposables-based plant are considered to remain unaltered when compared to those of a conventional plant.

- **Validation:** The validation of a disposables-based versus a conventional process will differ due to performance qualification (PQ) and operational qualification (OQ):

- Reduced or no cost of validation for the cleaning, sterilisation and turnaround of process equipment when using disposable equipment. This argument is already used in the qualification of use of disposable containers. The challenge and hence costs of cleaning validation for more complex equipment such as membranes also bears on this factor.

- The cost of validation of linkages between equipment (sterile welding versus conventional sealed pipe joints) is likely to remain the same.

- Most pieces of equipment will come pre-validated from the manufacturer (OQ).

3.3.3 Model for evaluation of disposables-based capital investment

From the assumptions presented in the previous section it is possible to estimate values for the factors to be used in Equation 3.1 that translate conventional into disposables processing (f_1' to f_{10}').

The factor f_1' was assumed to be 0.2 considering that utilities costs account for less than 40% of the total equipment costs and that they are reduced by a factor of 50% since there is no need for a clean steam package, etc (see Table 3.2). This factor also assumes that all the process equipment is disposable, i.e. the capital investment required for process equipment is zero. This can be confirmed by Joly (1998), who presented a cost comparison of a bag with a tank at the scale of 200 L where the cost of hardware for the bag (bag holder) is shown to be 0.3% of the cost of the stainless steel tank.

The values assumed for the remaining conversion factors are summarised in Table 3.3, together with the resulting overall factors for disposables ($f_i f_i'$). A conversion factor of 1 was taken for the items whose cost was considered to remain unchanged.

A "Lang" factor is then obtained for the disposable plant, $L_{disp} = 4.75$ based on the equipment cost for a conventional option. This value is 58% of that for conventional bioprocessing plants ($L_{conv} = 8.13$), thus indicating that the capital investment of the disposables-based plant will be reduced in the same proportion. In strict terms the

Lang factor of the disposable plant should be based on the equipment costs of the same plant. This factor would then be 23.7. It was decided not to follow this approach due to the high degree of uncertainty regarding the evaluation of equipment capital costs of a disposables-based plant, which would then be carried over to the overall capital investment.

i	Description	f_i'	$f_i f_i'$
1	Equipment and utilities	0.2	0.2
2	Pipework and installation	0.33	0.3
3	Process control	1	0.37
4	Instrumentation	0.66	0.4
5	Electrical power installation	1	0.24
6	Building works	0.8	1.33
7	Detail Engineering	0.5	0.39
8	Construction and site management	0.75	0.3
9	Commissioning	1	0.07
10	Validation	0.5	0.53
	Contingency factor (c')	-	1.15
	"Lang" Factor	-	$L_{disp} = 4.75$

Table 3.3 Capital investment factors for conventional (f_i) and disposables-based (f_i f_i') bioprocessing plants and corresponding "Lang" factors (Novais et al., 2001).

3.3.4 Sensitivity analysis

The study so far looked at a situation where a new building at a new site needs to be constructed. It might however be the case that the company already owns a building and simply wishes to convert it. In this case the building costs are significantly lower and will have a different impact in the way the two options compare. Sensitivity analysis was done for a range of building costs in the conventional case model and considering that these are reduced by 20% with disposables (Figure 3.1).

Contrary to what could be expected, the absence or reduction of building costs has a limited effect on the way the two options compare. In the best scenario, an unlikely case where no construction is required (building costs = 0), the capital investment of the disposables-based plant is still 52% of that of the equivalent conventional plant.

Adding the fact that the savings on building costs achievable in a disposable option may be even less than 20% when a building already exists, the effect of building costs is reduced still further.

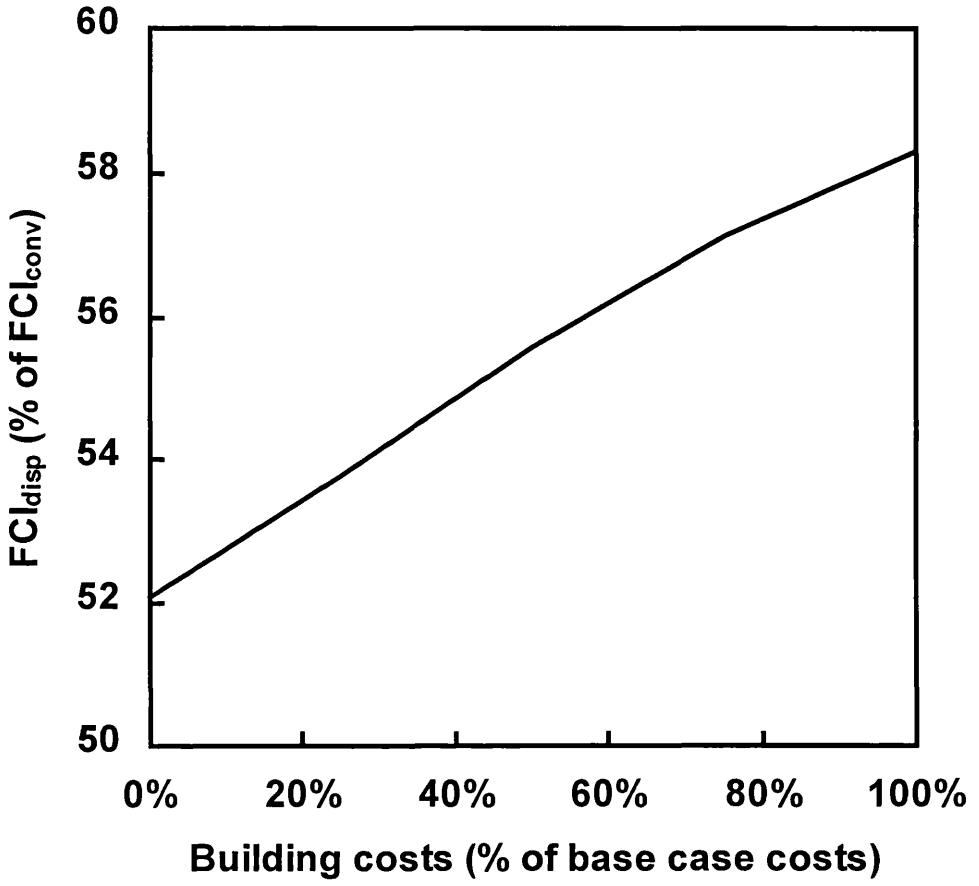


Figure 3.1 Sensitivity analysis to the effect of different building costs on the capital investment of a disposables-based plant (expressed as a percentage of that of the equivalent stainless steel plant). Note: it was assumed that building costs are reduced by 20% in a disposables-based plant.

Sensitivity analysis to the conversion factors (f_i') used in section 3.3.3 was done in the form of a worst/best case scenario study. Table 3.4 shows the worst and best case values of the different conversion factors, based on the assumptions described in section 3.3.2.

The worst case scenario for equipment and utilities costs is that these would only be reduced by 50%. This would correspond to an extreme case where for example the fermenter cannot be disposable: the presence of a stainless steel piece of equipment will require the existence of all the basic utilities, even if at a small scale. A similar reasoning led to the range of variation of pipework and installation costs indicated in Table 3.4.

The cost of process control was allowed to vary up to 1.2 times that of the conventional plant. This would be in an extreme case where there is a complete conversion to non-invasive monitoring leading to greater costs in computing hardware for data interpretation. In that situation instrumentation costs would not be reduced at all, with a conversion factor of 1.

The variation of building costs has been studied in detail in section 3.2, and consequently a narrower range could be used here. However the building conversion factor was allowed to vary up to 1, taking into account an extreme case where the company does not wish to take the risk of building a different style of facility.

The cost of detailed engineering is most certainly reduced for a disposable and hence intrinsically simpler design. The extent of this reduction is however unknown, and it was considered that the worst case scenario (minimum achievable) for this item would be a reduction of 20%.

It was considered that validation costs will not be more than 80% of those of a conventional plant. In fact approximately 80 to 85% of the validation costs correspond to the preparation of SOPs, staff training, etc (Doyle, 2000). As no cleaning and sterilisation will take place these tasks will not be needed in a disposables-based environment. Even if there is only a 25% reduction on these elements of the validation costs they still account for an overall 20% saving in validation.

	Conversion factor (f_i')		
Capital investment item	Base Case	Best Case	Worst Case
Equipment and utilties	0.2	0.1	0.5
Pipework and installation	033	0.1	0.7
Process control	1.2	0.5	1
Instrumentation	0.66	0.4	1
Building	0.8	0.6	1
Detail engineering	0.5	0.4	0.8
Validation	0.5	0.3	0.8

Table 3.4 Base case and best and worst values for selected conventional to disposable conversion factors (f_i').

Figure 3.2 shows that even in extreme cases the capital investment of the disposables-based plant is within +/- 10% of the base case. The most critical conversion factors are those governing the costs of the building, validation, and pipework and installation.

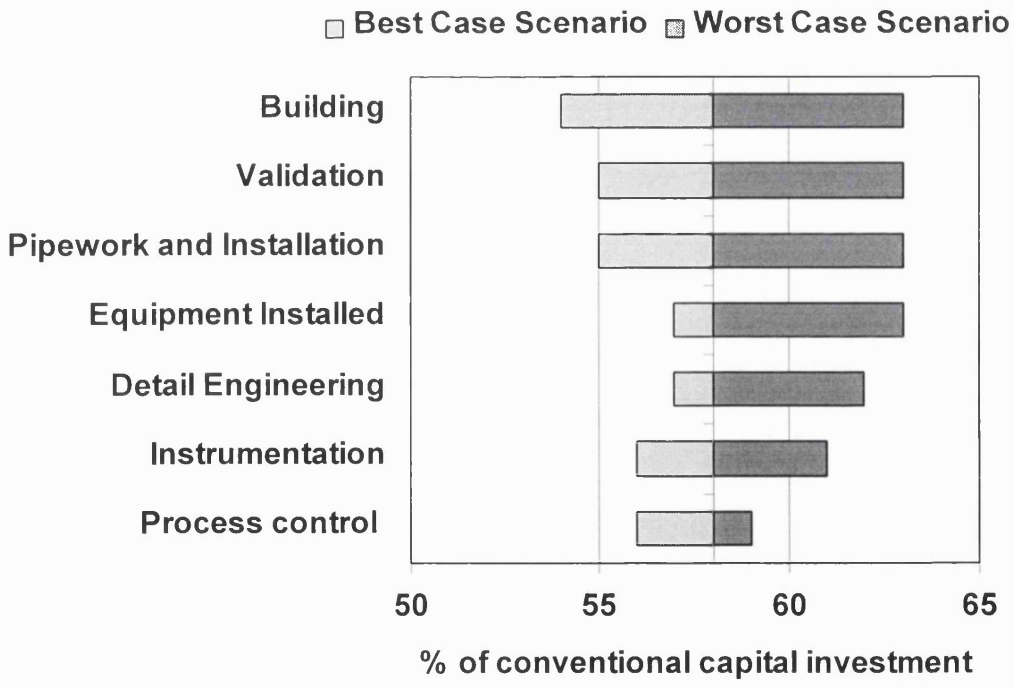


Figure 3.2 Sensitivity diagram for the conversion factors affecting the capital investment of the disposables-based plant. The y-axis crosses the x axis at the point corresponding to the base case (disposables-based capital investment is 58% of that of the equivalent conventional plant).

3.3.5 Other methods

A similar capital investment analysis can be made for the model presented in Table 2.4 of section 2.2, based on Petrides et al. (1995). The same assumptions and conversion factors were used. The sole difference is that instrumentation and control costs are not indicated separately in this model. Since previously the cost of control was thought to remain unchanged and that of instrumentation to be reduced by a factor of 0.66, the joint effect was calculated to be 0.8, taking into account the relative weights of the two items as shown in the model of Table 2.5 (Chapter 2). According to this model the total Lang factor for the disposables-based route is estimated at 3.97 (Table 3.5). This results in an estimate of the capital investment for the disposables-based facility at 53% of that of an equivalent plant based on conventional stainless steel equipment. This value is close to that calculated in section 3.3.3.

ITEM	f_i'	$f_i f_i'$
Direct Costs (DC)		
Purchased Equipment	**0**	**0**
Installation	0	0
Instrumentation	0.8	0.40
Process Piping	0.33	0.12
Electrical	1	0.10
Buildings	0.8	0.68
Yard Improvement	1	0.15
Auxiliary Facilities	0.5	0.30
Insulation	1	0.03
Indirect Costs (IC)		
Engineering	0.5	0.51
Construction	0.75	1.07
Contractor's Fee	0.75	0.25
Contingency (10% of DC+IC)	-	0.36
LANG FACTOR		**3.97**

Table 3.5 Conventional to disposable conversion factors (f_i') and factors for estimating capital investment items of a disposables-based plant based on the cost of the delivered equipment of the equivalent conventional plant (modified from Petrides et al., 1995).

A different approach could have been be taken for the calculation of the indirect costs and contractor's fee of the disposable plant: instead of estimating f_i' for these items, $f_i f_i'$ can be estimated based on the direct costs of the disposables-based plant, as was done for the contingency factor in the two previous models. Indeed the model indicates engineering and construction costs to be 25 and 35% of the direct costs respectively, and the contractor's fee to be 5% of direct plus indirect costs (Table 2.4, chapter 2). However it is unlikely that the indirect costs will be related to the direct costs in the same proportion as in the conventional plant, and so this method was not used.

The same analysis was done for the chemical engineering model presented in table 2.3 of chapter 2 (adapted from Peters and Timmerhaus, 1991). L_{disp}=2.18 compared to 5.1 for the conventional route (Table 3.6), indicating that the capital investment for the disposables-based facility is approximately 45 % of that of the conventional plant. This estimate is lower than the previous ones and will not be considered since the nature of the plant on which it is based does not include important bioprocessing features that impact costs significantly.

ITEM	f_i'	$f_i f_i'$
Direct Costs		
Purchased Equipment - Delivered	0	0
Purchased Equipment - Installation	0	0
Instrumentation and Controls - Installed	0.8	0.14
Piping - Installed	0.33	0.22
Electrical - Installed	1	0.11
Buildings - Including Services	0.8	0.36
Yard Improvements	1	0.10
Service Facilities - Installed	0.5	0.35
Land (if purchase is required)	1	0.06
Indirect Costs		
Engineering and Supervision	0.5	0.17
Construction Expenses	0.75	0.31
Contractor's Fee	0.75	0.16
Contingency factor (c')	N/A	1.1
LANG FACTOR	-	**2.18**

Table 3.6 Conventional to disposable conversion factors (f_i') and factors for estimating capital investment items of a disposables-based plant based on the cost of the delivered equipment of the equivalent conventional plant (modified from Peters and Timmerhaus, 1991).

3.3.6 Discussion

The capital investment of the disposables-based plant was evaluated to be 58% of that of the conventional plant. The method developed from the model from Petrides et al. (1995) gives a very close result, 53% of conventional, validating the approach taken.

The method developed from a chemical engineering model was used in order to evaluate the impact of a non-biotech model. Although not very different from the results above, the result obtained with this model (45% of conventional) is outside the range indicated by the sensitivity analysis.

The assumptions were validated through sensitivity analysis (section 3.3.4). The worst/best case scenario analysis shows that none of the variables affects the capital investment ratio (disposables/conventional) by more than +/- 10%. For example, the base case considered that the building costs would be reduced by 20% although the

analysis in section 3.2 had indicated this reduction to be higher, at 30%. The impact of changing this value is however very small: with this latter figure the capital investment of the disposables-based plant becomes 56% of that of the conventional equivalent plant. In fact, despite being the variable with the highest impact in Figure 3.2, it's effect is still less than a +/- 10% change on the capital investment ratio.

It is therefore possible to say with confidence that the use of disposable equipment allows for an approximate 40% saving in the capital investment required to build a new plant.

3.4 Running costs

3.4.1 Introduction

The costs of the disposable option can be predicted from considerations on how each category of costs varies when compared to the equivalent conventional option. It is expected that materials (raw materials and consumables, including disposable equipment) will increase significantly and that utilities costs and depreciation costs will be reduced, the latter due to the lower capital investment involved.

3.4.2 Assumptions

Several assumptions have to be made for the estimation of each category that constitute the running costs of a disposables-based plant (developed from Novais et al., 2001):

- **Operating labour:** Costs associated with cleaning and sterilisation will be decreased but staff will be needed to assemble/disassemble components, as well as to operate sterile welding systems. Labour costs associated with in-house media and buffer preparation will be decreased. Considering that in a bioprocessing plant there are operators whose tasks are exclusively associated with production support, which includes buffer and media make-up, equipment assembly, washing and autoclaving, etc (D. Sherwood, 1999) it can be concluded that there may well be a reduction in the staff requirements of a disposable plant. At Lonza Biologics, in 1999, production support constituted 25% of the people in operation (operating labour + supervision). Approximately 75% of the people in this team were operators for

buffer and media make-up, equipment assembly, washing, autoclaving, etc. For instance a 50% reduction on the number of these operators would lead to a 10% reduction on the overall number of operators.

- **Supervision, QC/QA and plant overheads:** These costs are likely to remain the same as in the conventional plant. The question is whether they do so in proportion with the operating labour of the disposable plant or stay identical to those of the conventional plant. Certainly at least supervision costs should maintain the same proportionality with operating labour.

- **Materials:** Costs associated with raw materials will be increased, as these will be bought as preformulated media and buffers and supplied pre-sterilised in bags. The cost of these items is higher so as to include the expense of the containers and the operating costs incurred by the supplier for the preparation and sterilisation of the media and buffers. The cost of disposable items (e.g. membranes, vessels, chromatographic media, pipework, etc) is also included in this category and will become a major factor.

- **Utilities:** Costs associated with steam and cleaning requirements will be reduced or even absent, therefore strongly reducing utilities running costs.

- **Depreciation:** This cost should be reduced as it is only associated with the process plant capital investment, which is lower for a disposables-based plant.

- **Other (patents, royalties, waste treatment, etc):** The remaining costs are possibly unaffected with, for example, the high effluent treatment costs for cleaning agents associated with the conventional option being offset by the increased costs for solid waste treatment of the disposable option.

3.4.3 Running costs of disposables-based plants

3.4.3.1 Bacterial process

Equation 2.7 in Chapter 2 can be altered in order to accommodate the assumptions outlined in the previous section, becoming (Novais et al., 2001):

$$RC_{disp} = RC_{conv} \sum_{i=1}^{5} x_i y_i$$

Equation 3.2

where RC_{disp} is the running cost of the conventional plant and y_1 to y_5 are factors which convert the individual conventional running cost fractions into disposables-based ones.

The factors y_1 to y_5 can be estimated from the assumptions in section 3.4.2 and are shown in Table 3.7. As a first approach it was assumed that the disposables-based plant has the same staff requirements as its conventional equivalent (y_1=1). Novais et al. (2001) noted that there is a 16-fold increase in the running costs associated with all materials and consumables for a bacterial process in a disposables-based approach, hence as a first approach y_2=16 (case study shown in Chapter 4). The running costs associated to utilities were assumed to be halved (y_3=0.5). Depreciation costs are reduced as a result of the lower capital investment involved as shown in section 3.3.3, that is y_4=0.6 and other costs are assumed to remain unchanged (y_5=1).

According to the analysis in Table 3.7 the running costs of a disposable biopharmaceutical plant are approximately 70% higher than the equivalent conventional costs.

i	item	y_i	$x_i y_i$
1	Labour costs	1	0.14
2	Materials	16	0.93
3	Utilities	0.5	0.07
4	Depreciation	0.6	0.11
5	Other	1	0.47
	TOTAL	-	1.72

Table 3.7 Disposable running costs factors derived from a cost distribution presented by Datar, et al. (1993) for a bacterial process. The item "Other" includes costs such as patents and royalties, waste, indirect manufacturing expenses, etc.

3.4.3.2 Mammalian cell process

The same conversion factors as above were used with the breakdown derived from that presented by Beck (2000) shown in section 2.4.4, except for the materials costs factor (y_2). Farid et al. (2000a) have found that the cost of raw materials + direct utilities increases 3 fold for a disposable plant. This corresponds to a 3.5 to 4-fold increase in the materials costs alone. However in that case study it was assumed that the stainless steel plant would also make use of ready-made media and buffers, thus decreasing the gap between the two scenarios. In this case it will therefore be assumed that materials costs increase by 5-fold. Utilities costs were separated from other direct costs for ease of calculations.

i	item	y_i	$x_i y_i$
1	Operating labour	1	0.12
2	Materials	5	1.30
3	Utilities	0.5	0.03
4	Other direct costs	1	0.09
4	Fixed charges	0.6	0.17
5	Plant overheads	1	0.18
	TOTAL	-	1.89

Table 3.8 Disposable running costs factors derived from a cost distribution presented by Beck (2000) for a mammalian cell process. The item "Other" includes costs such as patents and royalties, waste, indirect manufacturing expenses, etc.

According to the analysis in Table 3.8 the running costs of a disposable biopharmaceutical plant are approximately 90% higher than those of the equivalent conventional plant.

3.4.4 Sensitivity analysis

As the running costs of the disposables-based plant are evaluated based on assumptions, a worst/best case scenario study was performed to evaluate the robustness of the results obtained for a bacterial process in section 3.4.3.1. The conversion factors (y_i) and hence the assumptions made on 3.4.2 were the variables studied (see Table 3.9).

Labour costs were considered unchanged in the base case but, as it was said, they may decrease slightly. Here they were allowed to decrease down to 80% of the conventional costs. Likewise it is very unlikely that these costs should increase, as the only way that could happen would be if assembling / disassembling operations turned out to be more demanding than those associated with cleaning and steaming. Still the worst case scenario was taken for y_1=1.1.

Materials costs is the most critical variable because not only there is very little information available on these costs, they may also be case-specific. The cost of the materials will additionally depend on the quality of the materials chosen, as will be shown on Chapter 4. This means that even the worst and best cases chosen for this analysis are somewhat subjective.

The cost of utilities is no doubt reduced, the only uncertainty is the extent of reduction. The corresponding conversion factor was therefore allowed to vary from 0.3 to 0.8.

The change in depreciation costs is well known, directly proportional to the variation of the capital investment thoroughly studied in section 3.3. Consequently a small variation range was used for this factor. Also indirect manufacturing expenses include maintenance costs, so this factor may well be less than 1 in a disposables-based plant. Finally waste treatment is a difficult variable due to the opposite effects of increased solid waste vs. reduced liquid waste, and is therefore varied over a wide range.

	Conversion factor (y_i)		
Running costs item	Base Case	Best Case	Worst Case
Labour	1	0.7	1.1
Materials	16	8	20
Utilities	0.5	0.3	0.8
Depreciation	0.6	0.5	0.7
Indirect Manufacturing Expenses	1	0.5	1.1
Waste treatment	1	0.5	2

Table 3.9 Base case and best and worst values for conventional to disposable conversion factors (y_i). Indirect manufacturing expenses and waste treatment are both part of the item other (y_5) but are here presented separately.

The results in Figure 3.3 indicate that, apart from materials costs, the variables studied impact the running costs marginally (less than +/- 10% of the base case). In particular it is interesting to note that the variation in labour costs has a very small impact on the running costs. This indicates that it is not critical to determine this variable with high accuracy for a disposables-based manufacturing unit. Also the range of variation chosen for waste treatment costs does not impact the running costs by more than +/- 10%. This means that despite the high degree of uncertainty associated with this variable, its careful determination will not be key. On the other hand materials costs clearly need to be studied in more detail in order to obtain a reliable value for the running costs of the disposables-based plant.

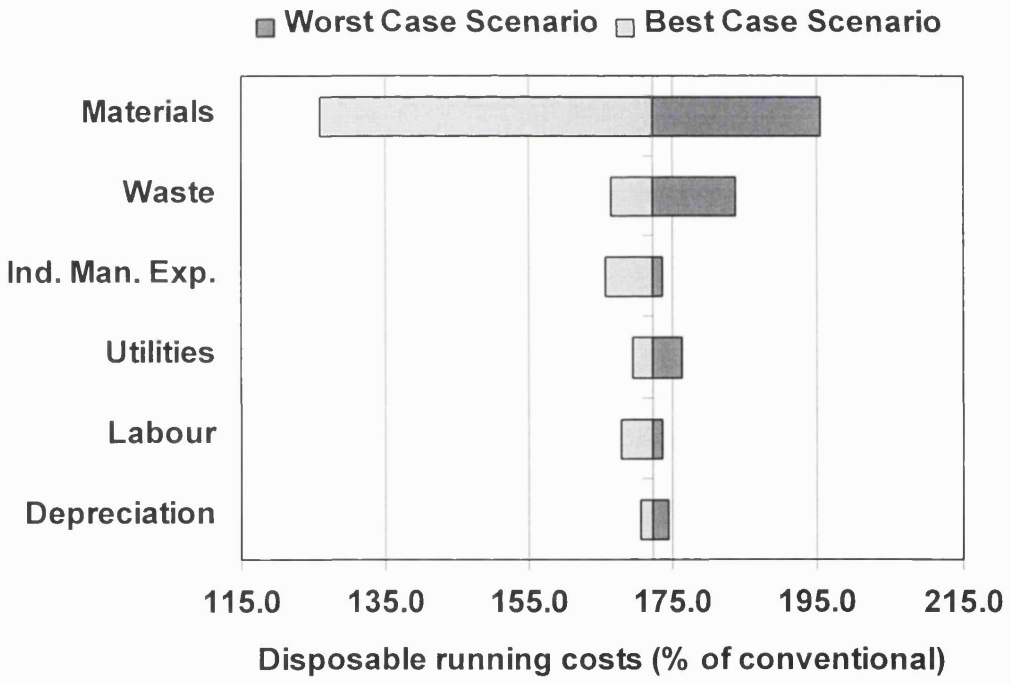


Figure 3.3 Sensitivity diagram for the conversion factors affecting the running costs of the disposables-based plant. The y-axis crosses the x axis at the base case (disposables-based running costs are 172% of those of the equivalent conventional plant)

Figure 3.4 shows more information on the effect of materials costs over the disposables-based running costs. In order for the disposables-based running costs to be identical to those of the conventional option (RC_{disp} = 100% RC_{conv}), the cost of materials has to increase only 3.5 fold. Conversely, if materials costs are 2 times higher than predicted, i.e. 32 fold higher than those of the conventional option, this leads to a 50% increase of the running costs compared to the base case. Given the clearly significant impact of materials costs this factor will be the subject of a thorough analysis. Examination of the effect of materials costs will be performed in Chapter 4 for a representative case study.

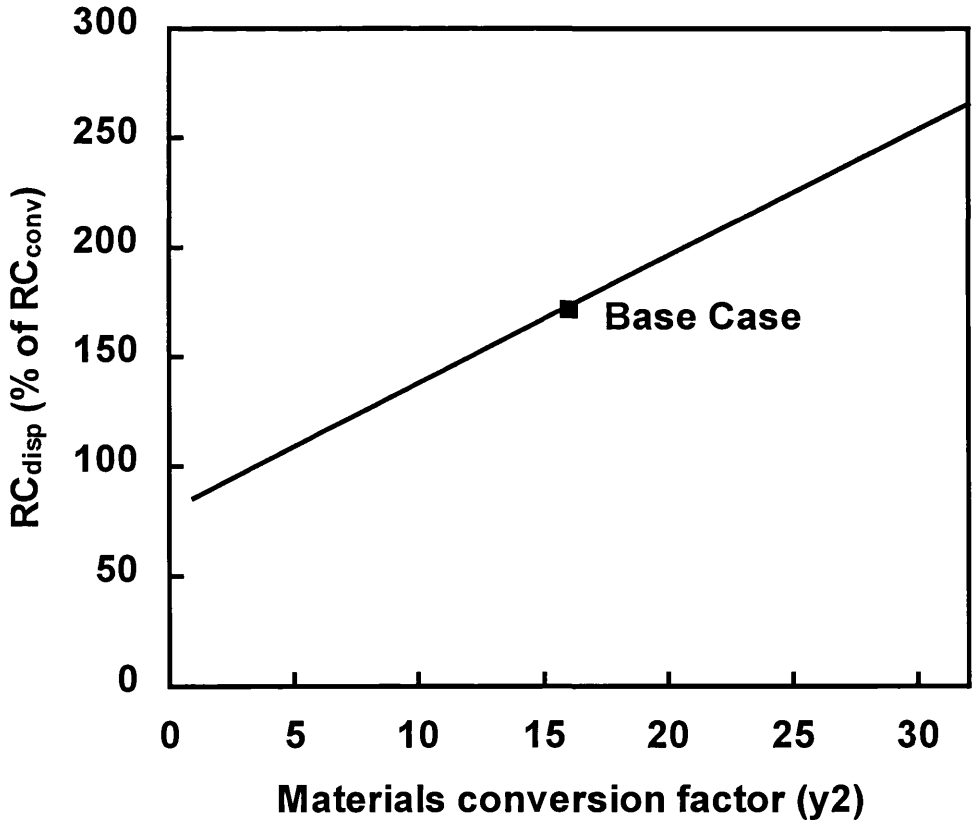


Figure 3.4 Analysis of sensitivity of the disposables-based running costs to the materials costs conversion factor (y_2).

The sensitivity analysis for the mammalian cell model in section 3.4.3.2 shows a similar trend (analysis not shown) although the impact of the increase in materials costs is greater, due to the higher proportion these costs represent. Materials costs need therefore to be evaluated very carefully in order to obtain a suitable comparison between the two modes of operation.

3.4.5 Other running costs models

The chemical engineering model (section 2.4.2) was also used for comparative purposes. This model (Equation 2.5) can be reformulated to express the fixed running costs (FC_{disp}) of the disposables approach:

$$FC_{disp} = OL_{conv} \sum_{i=1}^{4} g_i g_i' + FCI_{disp} \sum_{i=1}^{4} h_i h_i'$$

Equation 3.3

where g_i' and h_i' are the factors that convert the conventional items into disposables ones.

As noted in section 3.4.2 it is likely that operating labour and supervision maintain the same proportionality in a disposables-based plant, so $g_1' = g_2'$. The costs for QC/QA and plant overheads are most likely the same as in conventional plant and therefore $g_3' = g_4' = 1$.

The same proportion should remain between capital investment and tax, insurance and depreciation, so $h_1' = h_2' = h_4' = 1$. The factor h_3' may be lower than 1 if maintenance is mainly related to the equipment rather than plant itself. Still this factor will be taken as 1, as in the previous sections.

Equation 3.3 can therefore be altered to take these arguments into account:

$$FC_{disp} = OL_{disp} \sum_{i=1}^{2} g_i + OL_{conv} \sum_{i=3}^{4} g_i + FCI_{disp} \sum_{i=1}^{4} h_i$$

Equation 3.4

with $OL_{disp} = g_1' \times OL_{conv}$.

Assuming that a disposable plant has the same operating staff requirements ($g_1' = 1$), the variation in the fixed operating costs will be exclusively due to the decrease of the FCI- dependent terms (tax, insurance, maintenance and depreciation), which in turn are directly related to the decrease of the fixed capital investment. The decrease of the fixed costs will therefore be between 0 and 40% depending on the relative weights of the OL-dependent terms vs. the FCI-dependent ones. This decrease is illustrated through a case study in Chapter 4.

The variable costs have to be estimated based on the process requirements and include raw materials, consumables (membranes, matrices, bags, flexible pipes, disposable valves) and utilities costs. In the same way as for fixed costs, the impact of the use of disposables on the variable costs can only be evaluated with a case study (see Chapter 4).

3.4.6 Discussion

The running costs of the disposables-based plant were evaluated to be approximately 70% higher than those of an equivalent plant based on stainless steel equipment. However, as seen in section 3.4.4 this result is strongly dependent on the variation of the cost of materials, a variable for which not much information is yet available. Additionally if the cost of materials varies widely from case to case it may be that a robust model cannot be developed for the evaluation of the running costs of a disposable plant.

The remaining variables had a very small impact on the ratio of the running costs. This is particularly reassuring for the case of the waste treatment costs where some uncertainty exists. Here even the wide variation range studied did not affect the running costs ratio by more than +/-10%. Also the very small impact of labour costs precludes the need for a detailed analysis of the staff requirements of disposables-based plants.

Additionally a model specific to mammalian cell based processes (section 3.4.3) indicates that the running costs of such processes increase in a similar way to those of bacterial processes.

Finally a model based on a classical chemical engineering approach was also investigated for evaluation of running costs of disposables-based plants. This model cannot be used to compare the two options in a generic form because it is process-specific, but it will be evaluated later for the case study presented in Chapter 4.

3.5 Time to market

3.5.1 Introduction

Possibly one of the most important advantages of the use of disposables is a potentially earlier entry to market, which in turn improves the economic value of the project. Several factors can contribute to the reduction of time to market. On the one hand the reduced capital investment needed can lead to earlier decisions, like for example when to start building. Additionally the simpler nature of disposables-based plants together with a more straightforward validation may take construction out of the critical path. On the process development stages the use of disposables will reduce down time and turnaround time and hence allow a higher throughput of drug candidates (Farid et al., 2000b). The concept "fail fast, fail cheap" (Rosenberg, 2000) becomes a reality. Furthermore an early entry to market increases the use of patent life and generates a stronger position in the market.

3.5.2 Decision to build

It is crucial to choose the appropriate moment to start construction. If construction starts too early it will carry a high risk. Conversely starting construction too late will bring it onto the critical path to market. Nicholson (1998) reckons that it is difficult to stay off the critical path since the size of the capital investment necessitates intensive discussion leading to significant delays.

According to Hamers (1993) the decision to build has to be made at least 3 years before planned use. A survey of US biotechnology start-up companies indicated that 69% had taken the decision to build by the start of Phase III and 59% had actually

started construction by then. The equivalent figures for Phase II are 55 and 29% respectively.

The smaller capital investment associated with a disposable plant might enable an earlier decision to build since the loss in case of failure will not be so high. Conversely since construction times are shorter for disposables-based plants (see section 3.5.3 below) the start of construction may be delayed while keeping it off the critical path. As a result more information indicating the likely success and market size for the drug would be available at the time of building.

3.5.3 Construction time

Figure 3.5 shows a tentative evaluation of how the construction time may be reduced in a disposables-based approach. Several assumptions were made to evaluate how the length of each step will vary.

Firstly the time required to complete the conceptual design is thought to be unaffected as it involves steps required for either type of plant such as process flow diagram, equipment list, facility layout, etc (Boland, 1994). Detailed design (including design development) was considered to be reduced by 25% due to less complex construction drawings (civil, structural, architectural, mechanical, instrumentation, electrical) and construction specifications.

The actual construction time is also shorter, estimated to be reduced by 25% due to the simpler building required. The period required for equipment start-up is especially reduced (estimated at 50%) due to the absence of fixed equipment in the disposables-based plant.

Finally validation time should be reduced by at least 33% for the reasons that follow. Validation includes installation qualification (IQ), operational qualification (OQ) and performance qualification (PQ) amongst other steps (Baird and De Santis, 1994). Installation Qualification (IQ) consists in comparing the installed system with the design documentation. In general terms this step will still be present in a disposables-based plant, although in this case the IQ will apply to disposable systems.

Operation Qualification (OQ) ensures that the unit or system operates as specified. Where possible this will be the responsibility of the disposable equipment manufacturer. In a disposables-based plant it will therefore only be required for the fixed systems such as some of the utilities.

Performance qualification (PQ) will remain unchanged for the process, although it may be simpler for utilities, since there are fewer to be tested in a disposable approach. Systems such as purified water (PW), Water for injection (WFI) and steam require long PQ periods of sampling. These will not be needed in the disposable plant if PW and WFI are bought in bags instead of being made in-house.

Validation time will also decrease due to the absence of media and buffer preparation steps as these will be bought ready-made.

The overall effect of the time reductions predicted above lead to an overall saving of 13.5 months on a total 4 years construction time indicated for a conventional plant. This corresponds to a total 28% saving in construction time.

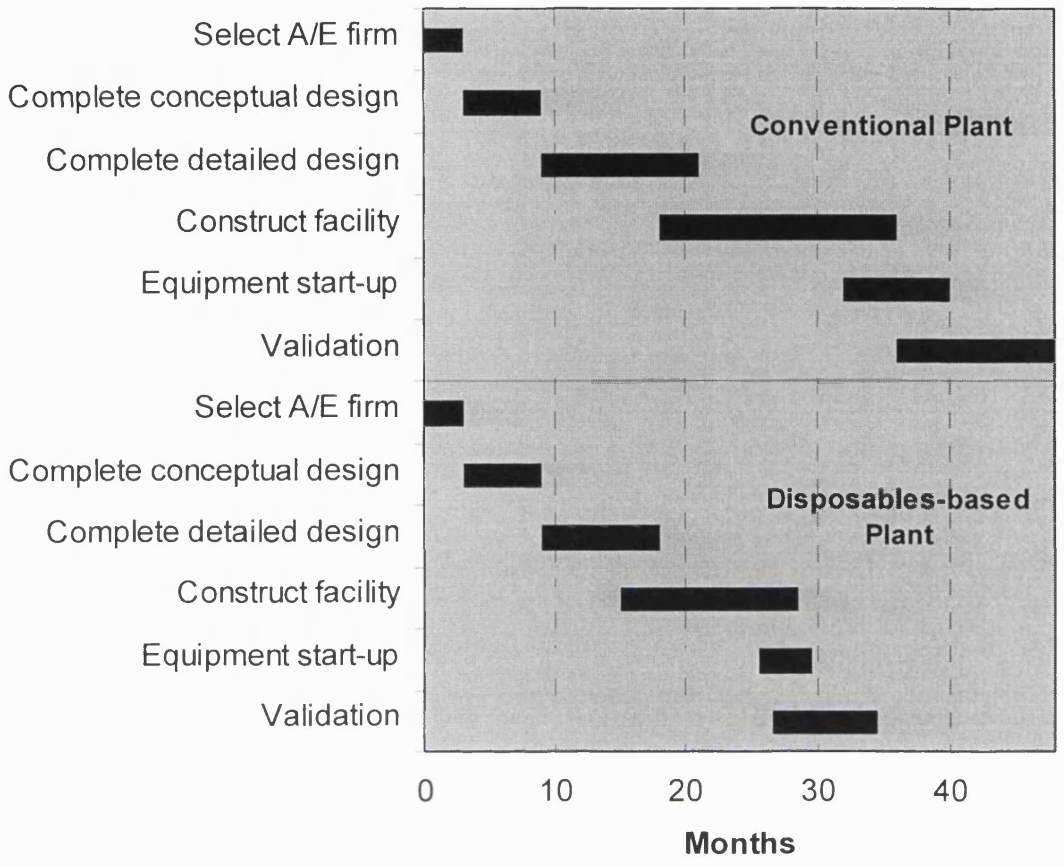


Figure 3.5 Time required to design, construct and validate a biopharmaceutical facility based on (top) conventional stainless steel equipment (adapted from Burnett et al., 1991) and (bottom) disposable equipment. (A/E: architectural/engineering.)

3.5.4 Process development time

The construction of the commercial facility is only one part of the path to market. More importantly the development stages include the clinical evaluation stages and the time for regulatory approval. In Figure 3.6 the timelines for R&D and preclinical development were kept unchanged in the disposables approach.

Phase I/II clinical studies take 12 to 14 months of which 2 months correspond to materialisation (Dennis, 1999). It was thought that even if there is some time saving at this stage it would be negligible. Phase III takes 24 months of which 3.5 months are for validation and 8 months are for manufacture. In this case it was deemed that the use of

disposables leads to a saving of at least 3 months due to simpler validation and shorter downtime between subsequent batches.

It was considered here that regulatory approval remains unchanged for a disposables-based process. This may initially not be the case while regulatory agencies get up to speed with the new implications associated with disposable equipment. However a good indicator of the longer term trend is that many companies make now use of disposable containers for buffer preparation without additional regulatory consequences.

According to Figure 3.6 there is an overall reduction of 1.5 years in a 10.5 years development time line. The average development time for a biopharmaceutical drug is now 7.8 years (Foo et al., 2001), so this should correspond to 13.5 months earlier entry to market assuming direct proportionality.

It has to be noticed that not all drugs will allow a disposable design at the commercial stage. Effectively there is a scale limiting factor as disposable containers are currently available up to 2500 L only, which would also constitute the maximum scale of the disposable fermenter. For drugs at a higher scale of operation the time savings brought by disposables will be smaller and associated exclusively with the clinical trial stages. This is provided the transfer from a disposable-based Phase III pilot plant onto a stainless-steel-based commercial plant can be done without major regulatory obstacles.

Another interesting way of examining the effect of the use of disposables on the time to market is to consider a portfolio of drugs, all associated with typical failure rates. As the use of disposables allows for a shorter changeover between subsequent drugs, the throughput can be higher and it may take less time to get one drug approved. Farid et al. (2000b) presented such a case study, where the use of disposables allows for a greater likelihood of achieving an annual throughput of at least 5 projects, i.e. a higher chance of getting more than a single product to market.

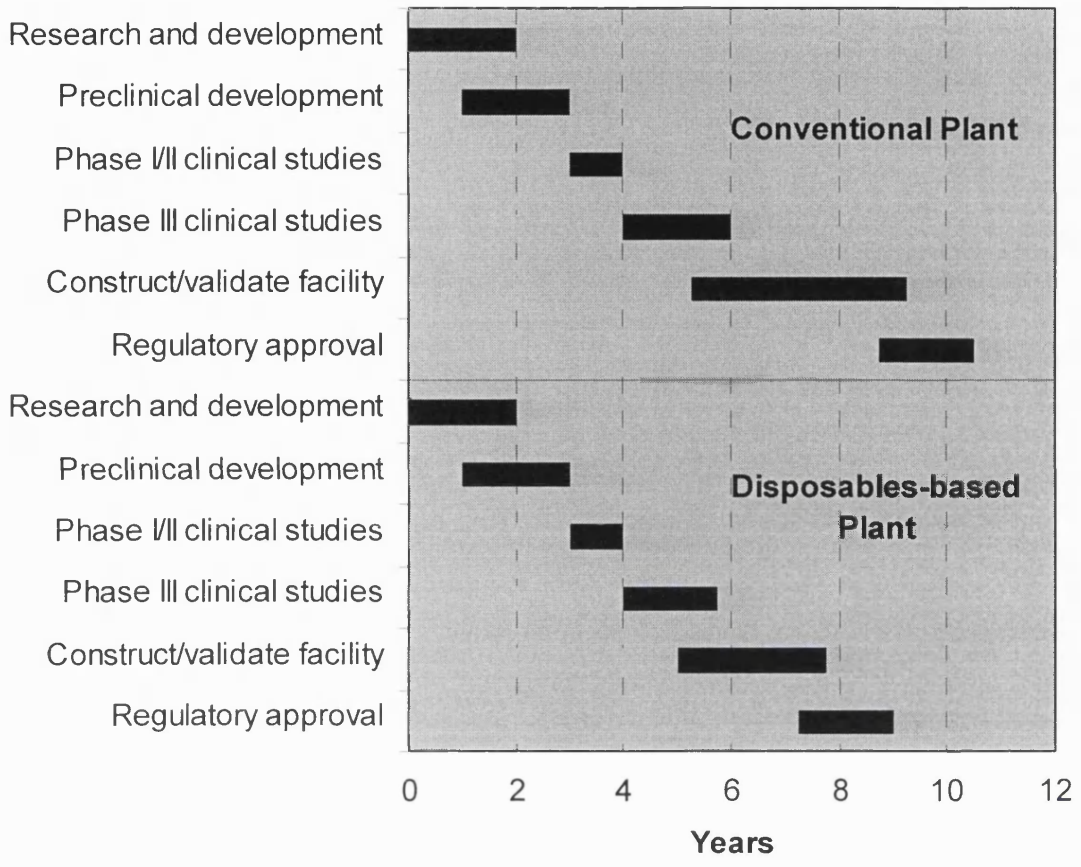


Figure 3.6 Time required to develop and licence a biopharmaceutical drug for (top) a conventional plant (adapted from Burnett et al., 1991) and (bottom) a disposables-based plant.

A further non-quantifiable effect has to do with cases where the only two viable options are to either build a disposable plant or to make recourse to a contract manufacturer. This can be the case for small/medium companies with limited resources. Given the present situation worldwide of a lack of manufacturing capacity and long waiting lists among contract manufacturers (Garber, 2001) the use of disposables can clearly offer further time savings.

3.6 Conclusions

The use of disposable equipment for the manufacture of biopharmaceuticals has many economic implications. On the capital cost side the area requirements, the complexity

and hence the cost of such plants are lower than those of equivalent plants based on stainless steel equipment. The reduction in building cost was estimated to be significant at approximately 30%. The absence of fixed equipment and many utilities signifies that the contents of such plants is also much simplified, with reduced fixed piping and associated validation. Overall the capital investment of a disposables-based plant was estimated to be 60% of that of a conventional equivalent plant.

The running costs are however significantly increased when operating in a disposable format, mainly as a consequence of new costs such as disposable equipment replacement and flexible tubing. This is despite the decrease in maintenance and in the operating costs of utilities. The increase in running costs was estimated to be around 70% for a bacterial process and 90% for a mammalian cell process. Both these figures were shown to be heavily dependent on the costs of disposable equipment, which in turn depend on the designs and construction materials chosen (see section 4.4.3). It is also likely that the prices of disposable equipment will go down once the market develops, thereby reducing the negative effect of this cost factor.

The overall effect of a decrease in capital investment versus the increase in running costs will have to be evaluated through a net present value evaluation and will be the subject of the next chapter (Chapter 4).

The time that can be saved when bringing a product to market was shown to be very significant, up to 1 to 1.5 years. This time saving can be translated into additional revenues, which can be of the order of £50 million per annum for a typical drug (Davidson, 1998).

The case study presented in Chapter 4 will seek to combine all the different factors studied in Chapter 2 and in this chapter, with the purpose of establishing an economic comparison between conventional and disposables-based technologies.

Chapter 4 Case study: Economic comparison of conventional vs. disposables-based bioprocessing

4.1 Introduction

Chapter 3 showed that in a disposables-based plant the capital investment is reduced and the running costs increase. However, in order to evaluate fully how these two factors combine, it is necessary to perform net present value (NPV) studies.

In this chapter a case study based on an *E. coli* fermentation was chosen to evaluate the NPV of the two options. This required the estimation of the capital investment (section 4.3.1) and of the running costs (section 4.3.2) for both processing alternatives. Sensitivity analysis was then performed to confirm the robustness of the results obtained (section 4.4) and finally the results are discussed in section 4.5.

4.2 Case Study

The case study presents a comparison between a conventional bioprocessing plant and its disposable equivalent. The system chosen has been the production of a Fab' antibody fragment of HuMAb4D5-8 using a recombinant *Escherichia coli* at a 300 L working volume fermentation scale of operation (see Chapter 1). Expression is mainly to the periplasmic space thus leading to the need to harvest the cells and submit them to a periplasmic release step. The cell debris/empty cells are then removed from the process stream and a first chromatographic or adsorption/desorption separation stage either for product capture (preferably) or for contaminant capture is achieved.

4.2.1 Stainless-steel based process

The conventional process would include two centrifugation steps as a means to achieve solid-liquid separation (cell harvesting and cell debris removal) and a final affinity chromatography step. The simplified process is represented in Figure 4.1.

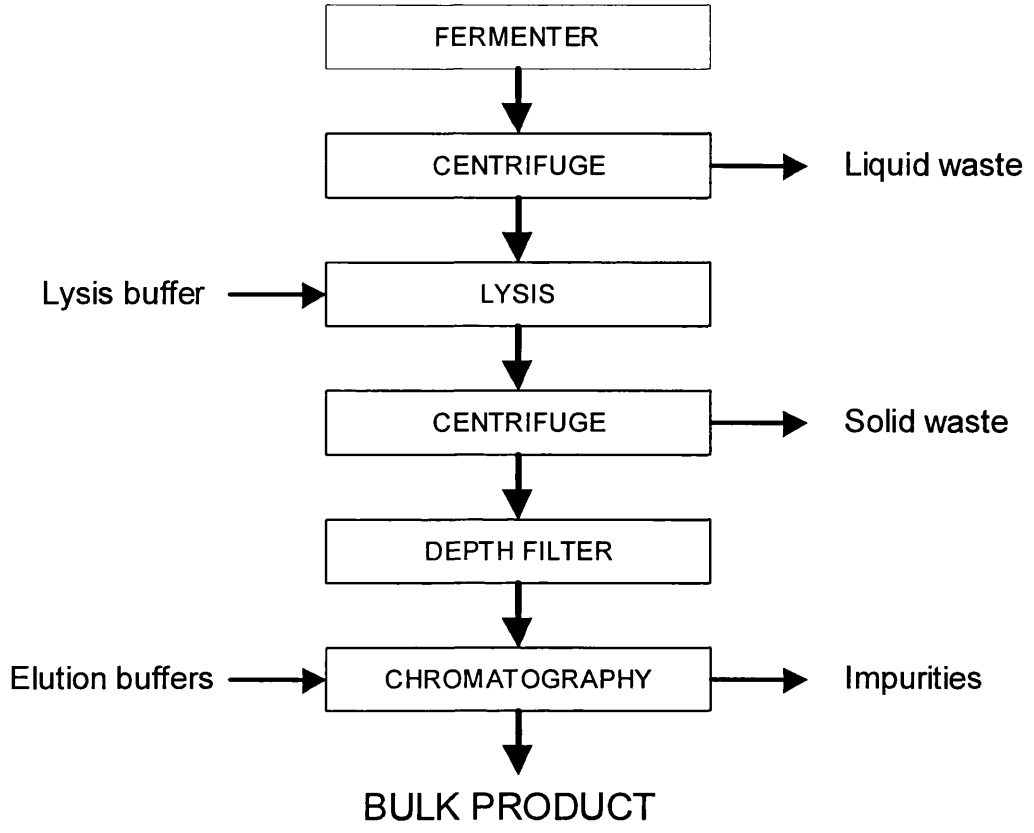


Figure 4.1 Simplified block diagram of a process making use of centrifugation for solid-liquid separation.

This process cannot be easily performed with disposable equipment due to the centrifugation steps (although it has to be noted that disposable centrifuges do exist, as indicated in Chapter 1). It was decided therefore to modify the process so as to exclude centrifugation steps, so that the same sequence and design can be sustained in the two modes of operation (stainless-steel and disposables).

The modifications to the process include the substitution of the centrifuges with tangential flow filtration modules. 0.1 μm PVDF (polyvinylidene fluoride) membranes were chosen for the cell harvesting step and 1000 kD regenerated cellulose or polyethersulfone (PES) membranes were chosen for the clarification step. The final product concentration step is achieved in the new process with 10 kD PES membranes (UF). Design calculations based on a 300 L fermentation were then performed in order to establish the appropriate equipment sizes and capacities and the main results are shown with the modified process block diagram in Figure 4.2. The complete mass

balance and design calculations are shown in Appendix 1 and the resulting equipment list is shown in Appendix 2.

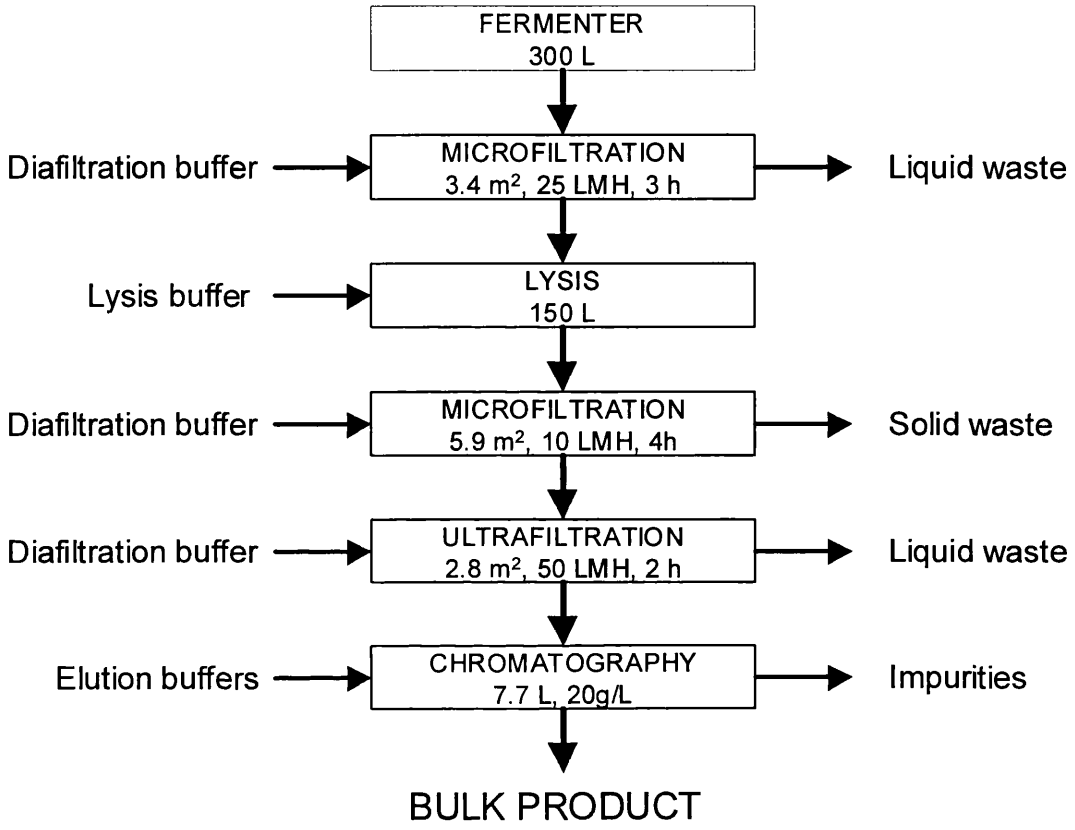


Figure 4.2 Block diagram of the modified process and some design values.

4.2.2 Disposables-based process

It was assumed that the disposables-based plant had a process sequence identical to that of the conventional plant. In particular the disposable process makes use of peristaltic pumps instead of invasive pumps (e.g. centrifugal pumps) and pinch valves for flow control. Heat transfer is achieved by disposable heat exchangers (see Chapter 1). Most importantly the fermenter is based on a plunging jet design as described in Chapter 1.

Given that bioprocessing bags are currently available up to 2500 L scale (Stedim corporate profile, 2000), operation at 300 L should not pose any major obstacle. The

detailed process scheme is shown in Figure 4.3A using conventional equipment and in Figure 4.3B using equipment configured for use in a disposable fashion.

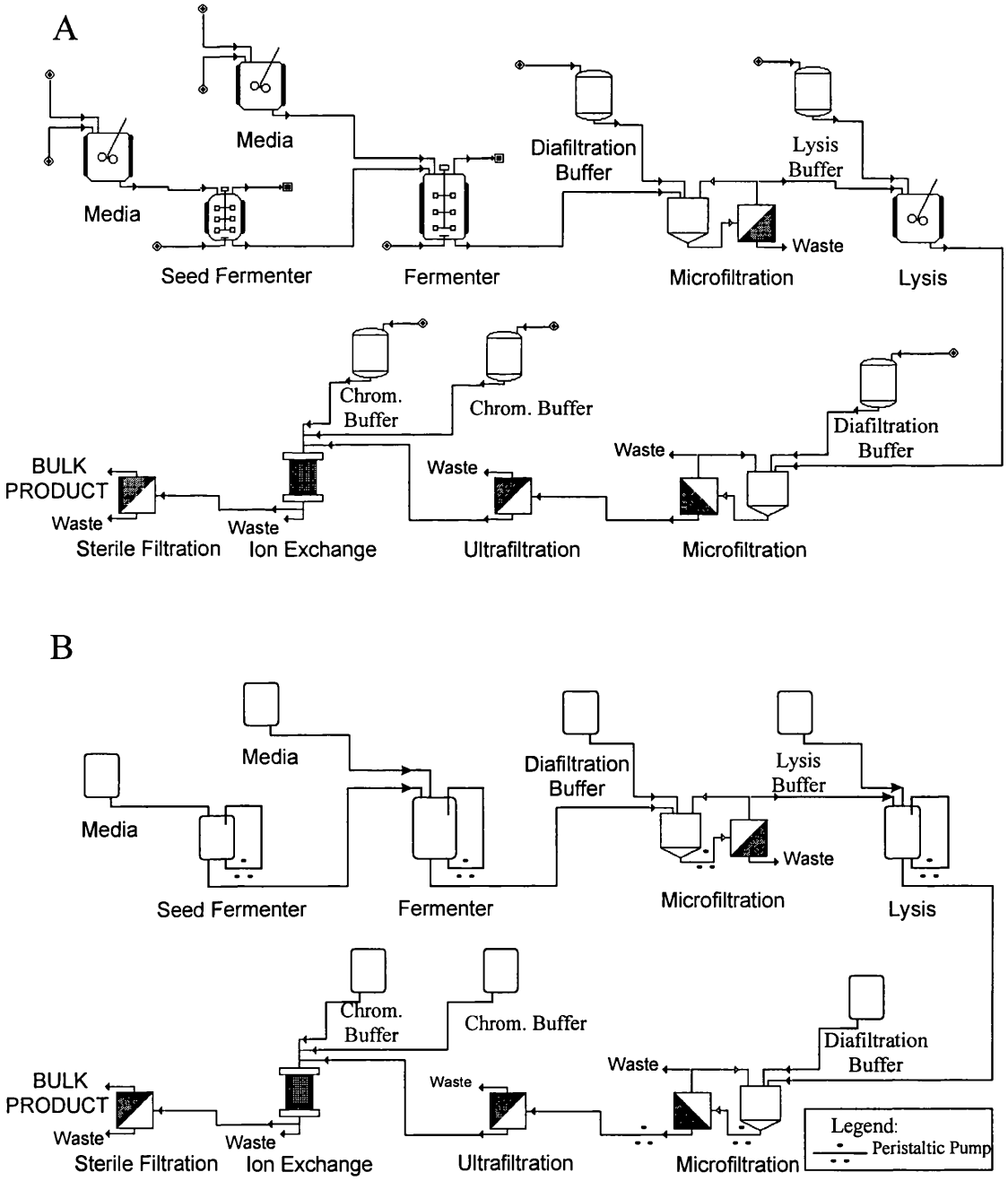


Figure 4.3 Process diagram of the case study process: E. coli production of an antibody fragment. A) conventional route and B) disposables-based route. In the latter case the process vessels are disposable bioprocessing containers and the fermentation is achieved with a plunging jet design.

The same yield per unit operation was considered for both processing options but the sensitivity analysis in section 4.4.4 will study the impact of a potential loss in performance in the disposable route. Both options were assumed to operate 48 batches/year for a project life of 8 years. Hence no account was taken of the potential for faster turn around of a process batch when using the disposables option. A sensitivity analysis considering the achievable time to market will also be performed later in section 4.4.3.

It was assumed that the decision to build the conventional manufacturing plant has to be taken by the start of Phase III clinical trials, that is approximately three years before entry to market. A similar constraint was applied for the disposables option. This assumption may result in a further underestimation of the benefits of disposables option which, through having a reduced capital expenditure, may enable such financially risky decisions (failure rates being as high as 1 in 3 at early clinical stages; Struck, 1994) to be made somewhat earlier.

4.3 Results

4.3.1 Capital investment

The conventional plant equipment costs (E_{conv}) were estimated in Appendix 2 for the process in Figure 4.3A and sum up to £1.57 million. The factors f_i and f_i' were estimated previously in sections 2.3.3 and 3.3.3 respectively. It is therefore possible to calculate the values of capital investment for both options FCI_{conv} and FCI_{disp} from equations 2.2 and 3.1. The conventional capital investment was evaluated at £12.8 million as opposed to £7.5 million for the disposable option, a 40% reduction as indicated in section 3.3.3 by the difference in the two Lang factors. Figure 4.4 shows the impact of a disposables-based approach on the different items that constitute the capital investment.

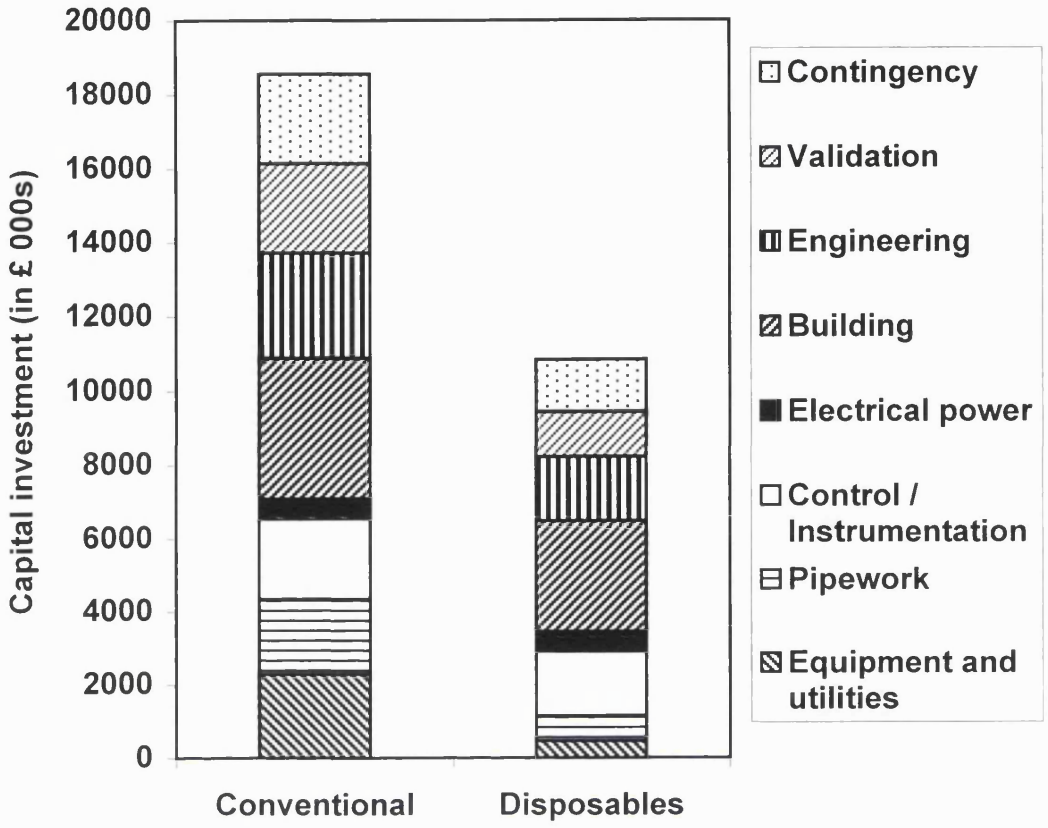


Figure 4.4 Breakdown of the capital investment cost of the conventional and disposables-based processes. The conventional plant breakdown was derived from standard figures for conventional bioprocessing plants (section 2.3.3). The capital investment breakdown of the disposables-based plant (see section 3.3.3) was obtained from the conventional one based on assumptions derived from the definition of disposable manufacture (section 3.3.2).

4.3.2 Running costs

The running costs breakdown factors x_1 to x_5 were presented in section 2.4.3 of Chapter 2. The cost of depreciation was estimated using the capital investment (FCI_{conv}) and a working life of 8 years for the plant. From there it is possible to calculate the cost of the other individual items of the running costs of the conventional plant through equation 2.7. The running costs of the disposables-based option were estimated through equation 3.2 and the factors y_1 to y_5 presented in section 3.4.3.1 of chapter 3. The resultant comparison for the running costs for conventional as opposed

to disposable operation are given in Figure 4.5, the running costs of a disposable biopharmaceutical plant being £14.7 million, i.e. 1.7 times higher than the equivalent conventional costs (£8.5 million).

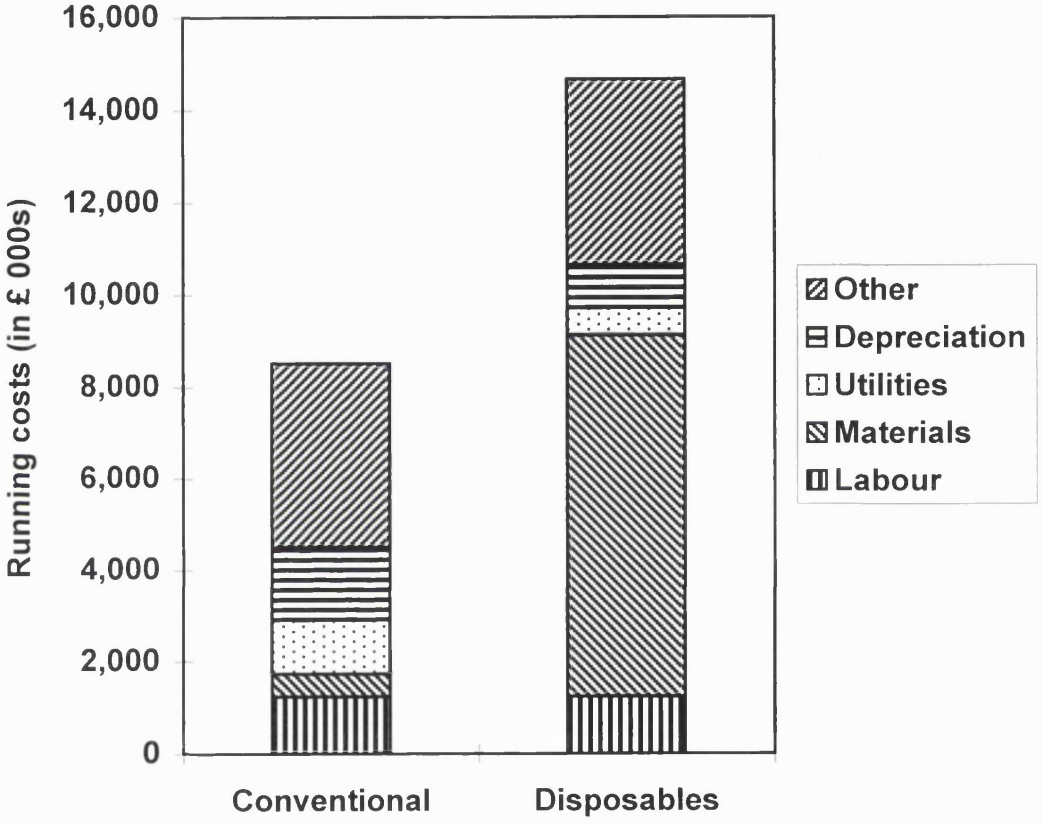
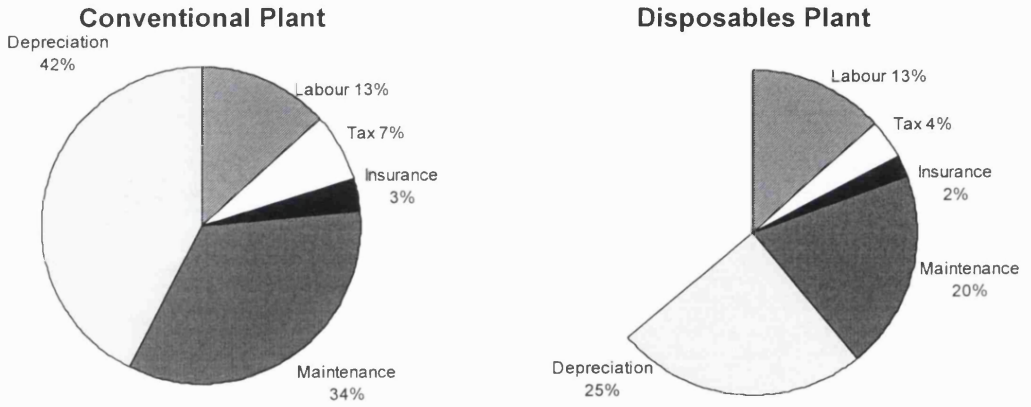


Figure 4.5 Breakdown of the running costs of the conventional and disposable processes (Novais et al., 2001). The conventional plant breakdown was based on a cost distribution initially proposed by Datar et al. (1993) for a bacterial process (section 2.4.3). The breakdown for the disposable option was calculated with the use of the factors y_1 to y_5 presented in section 3.4.3.1 and from the conventional capital investment value calculated in 4.3.1. The item "Other" includes costs such as patents and royalties, waste, indirect manufacturing expenses, etc.

The model originally developed for chemical engineering processes (sections 2.4.2 and 3.4.5) was also used for the calculation of the running costs for comparison purposes.

Considering the values of fixed capital investment calculated in the previous section 4.3.1 it is possible to calculate tax, insurance, maintenance and depreciation costs. The other fixed running costs items require an estimate of the operating labour force. Assuming the plant requires 8 operators working in a single shift the cost of operating labour sums up to £240 k per year. The cost of supervision, QC/QA and plant overheads can be worked out from this value. It is then possible to obtain a breakdown of the fixed costs (Figure 4.6), with a total annual value of £3.8 million for the conventional plant and of £2.4 million for the disposables-based plant.



£3.8 million p.a. £2.4 million p.a.

Figure 4.6 Fixed running costs breakdown for conventional and disposables-based plants, using the conventional total fixed costs as the basis (100%). The FCI based items (tax, insurance, maintenance and depreciation) of the conventional plant were obtained from the factors g_i in Table 2.7 (Chapter 2) and the capital investment calculated in section 4.3.1. The labour category includes operating labour, supervision, QC/QA and plant overheads and was obtained from the factors h_i and the calculated operating labour cost (8 single-shift operators, i.e. £240 k per year). The disposable plant breakdown was obtained with the disposable capital investment and considering the factors gi and hi are the same as in the conventional option.

As for the variable costs, each of the categories requires individual assessment based on the mass balance and equipment sizes. Table 4.1 shows the materials costs

calculated for both options based on the process requirements. The cost of the raw materials in the conventional plant was estimated from laboratory supplies catalogues (i.e. Sigma catalogue, 1998; BDH Catalogue, 1998) and by taking the costs for the largest available quantities and applying a discount factor of 3 was applied. For the disposable plant it was assumed that the media and the buffers are bought pre-prepared supplied in sterile containers and cost on average \$2.5/L (range of costs suggested in HyClone Europe price list, 1998). The running costs of the membranes and matrices in the conventional plant were calculated from membranes and chromatography manufacturers catalogues (Millipore (UK) Ltd. and Pharmacia) taking a conservative estimate that these are used 20 times before being replaced. In a disposable plant both membranes and ion-exchange matrices are disposed of after each batch. The item "other disposable equipment" includes all other equipment not specified above, such as bioprocessing containers and flexible pipes (HyClone Europe price list, 1998). This item is close to zero in a conventional plant. It was assumed that a disposable plant makes use of containers of the same volume as the stainless steel containers in the conventional plant and that it needs approximately 10 m of flexible tubing for each unit operation.

Variable Costs (\$k/year)	**conventional plant**	**disposables plant**
Raw materials	36	138
Membranes	58	1166
Matrices (IEX)	16	319
Other disposable equipment	0	191
TOTAL (Materials)	**110**	**1814**
Utilities	480	240
TOTAL (Materials + Utilities)	**590**	**2054**

Table 4.1 Annual variable costs estimates for both conventional and disposables-based routes (Novais et al., 2001). The cost of each item was obtained from the mass balance to the process and from the process flowsheet. The item "Other disposable equipment" includes bioprocessing bags and flexible pipes.

From Table 4.1 it can be noted that there is a 16-fold increase in the running costs associated with all materials and consumables in a disposables-based approach. This result constituted the basis for the choice of the value 16 for y_2 in section 3.4.3.1.

The running cost of utilities was estimated considering that a bioprocessing facility will have a utility bill of $320 per year and per m^2 of manufacturing area (M. Sawyer, personal communication) and that the case study facility will have an area of 1500 m^2. This is calculated as a fixed cost but will be considered a variable cost so as to be consistent with the initial model.

The outcome of this analysis is summarised in Figure 4.7 and shows that the overall running costs associated with a disposables option would differ by a factor of 0.9 to those of a conventional option, compared with a factor of 1.7 given above.

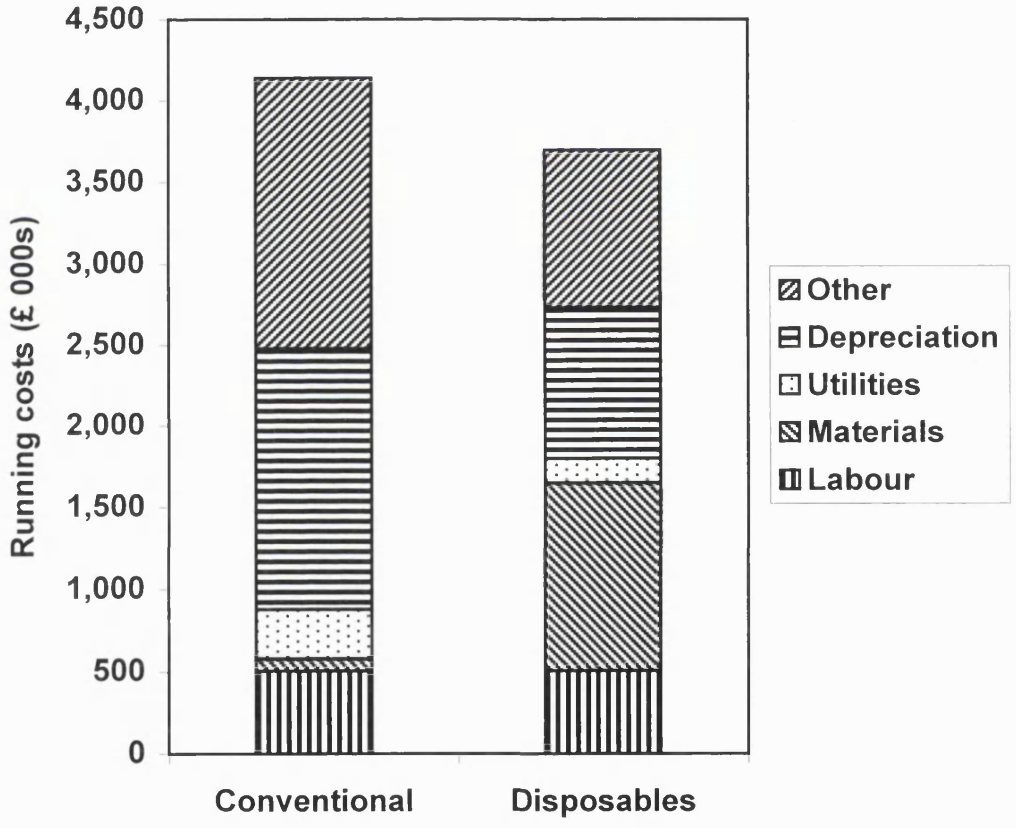


Figure 4.7 Breakdown of the running costs obtained for both conventional and disposables-based routes from a traditional chemical engineering model. The values of the different running costs items were obtained from Figure 4.6 and from Table 4.1. The conventional running costs were taken as the basis, set at 100%.

4.3.3 Net Present Value

To compare the disposables-based approach with the conventional one, the respective NPV were calculated according to Equation 1.2 (Chapter 1). In this equation it was considered that the investment was completed in year zero for both approaches. Both plants were considered to start operating at half capacity in year 1 (RC_1=1/2RC), achieving full capacity in year 2. Sales commences in year 3 (m=3, S_1=S_2=0). Taking the project life span as 8 years (τ=8) as specified in the description of the case study and the discount rate as r=0.2, Equation 1.2 becomes (Novais et al., 2001):

$$NPV = -FCI + \sum_{n=1}^{2} \frac{-RC_n}{(1+0.2)^n} + \sum_{n=3}^{10} \frac{S_n - RC_n}{(1+0.2)^n}$$

Equation 4.1

The net present value was obtained having set the annual sales for each case as 5 times the running costs of the conventional plant (Coopers and Lybrand company publication, 1997). The NPV of the disposables-based plant is estimated at £50.5 million, 26% lower than that obtained for the conventional option, £68.4 million. The results are summarised in Table 4.2.

Cost	Conventional	Disposable
FCI (£ million)	12.8	7.5
RC (£ million/year)	8.5	14.7
NPV (£ million)	68.4	50.5

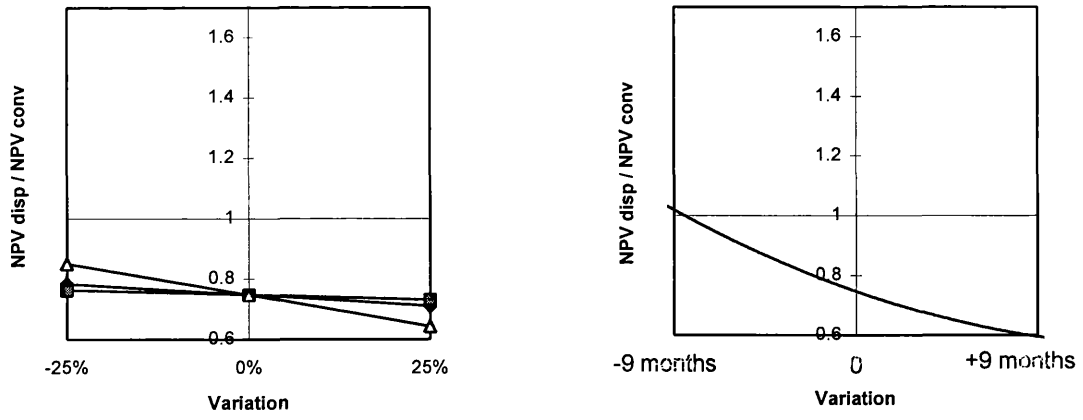
*Table 4.2 Economic analysis results summary (Novais et al., 2001). The fixed capital investment was obtained from the total cost of conventional equipment and from the "Lang" factors in Table 2.5 (conventional) and Table 3.3 (disposable). The running costs were obtained from Figure 4.5 and the fixed capital investment (*FCI*). The net present value (*NPV*) was calculated according to Equation 4.1 assuming annual sales of the Fab' antibody fragment to be 5 times the conventional running costs (Coopers and Lybrand company publication, 1997).*

4.4 Sensitivity analysis

4.4.1 Key variables

In order to evaluate the reliability of the comparison, sensitivity analysis was carried out for relevant variables in the disposables approach. The parameter chosen for the comparison was the ratio of disposable NPV over conventional NPV, which is >1 when the disposables-based option is financially the more attractive.

The variables studied included capital investment, staff costs and materials costs. Figure 4.8A shows the impact on the NPV ratio of a variation of +/-25% made to each of these disposable plant variables. (Note when the capital investment is varied, the depreciation costs are also affected as they are, by definition, directly related to the capital investment.) It can be seen that these three variables have only a slight impact on the NPV ratio. However the variation range for materials costs may be higher than +-25% as was indicated in Chapter 3. For that reason a separate study of the impact of the cost of materials was carried out in section 4.4.3.



A B

Figure 4.8 Sensitivity analysis: effect of a variation of +/-25% on crucial cost variables.. The variables studied were: a) Fixed capital investment (♦), Staff costs (□), Materials costs (Δ) and b) time to market(—). (Note: Only the disposable variables were varied.)

4.4.2 Time to market

Considering that opting for a disposables-based process may allow for earlier entry to market it is interesting to evaluate the impact this may have on the NPV. This is achieved using Equation 1.2 in Chapter 1 but considering that the start of manufacture and sales occurs earlier than year 'm'. This does assume that the life-span of the project (τ) is unchanged, i.e. that despite the earlier entry to market the product will be sold for the same length of time. That could result in an underestimation of the benefits. Non-linear least squares and interpolation were used to deal with fractions of years gained in time to market.

Figure 4.8B shows how a 9 months reduction or increase in entry to market with the disposables-based approach can affect significantly the NPV ratio. For example the NPV ratio is 0.74 when the time to market is the same but increases to 1.00 if disposables allows for a 9 months earlier entry to market.

4.4.3 Materials costs

The cost of the pieces of disposable equipment is crucial in the evaluation of this manufacturing alternative. The central costs are those of the membranes taking 64% of the total materials costs as can be seen from Table 4.1. The second most important cost is that corresponding to the matrix at 18% of the total costs.

Figure 4.9 shows the impact on the NPV ratio of using cheaper membranes. The first set of bars looks at the replacement of traditional membranes in the two UF steps of the process, i.e. lysate clarification and product concentration. As can be seen from the graph the NPV ratio reaches a plateau at approximately 0.93. There is a negligible difference between the impact of a price 10 or 100 times lower.

The case study considered that flat sheet membranes are used in both processing options. These high quality membranes are designed to be re-used and are therefore expensive with a price per area of approximately £1200 per m^2 (Millipore catalogue 1999). However other types of membranes can be used, namely disposable hollow fibre membranes developed for medical applications. The high production volumes of such membranes result in lower prices. For example disposable kidney dialysis cartridges manufactured by Nissho Nipro Corporation (distributed in the U.K. by

HyMed Healthcare Ltd.) are sold at £29 per 2.1 m^2 cartridge (cellulose triacetate). This corresponds to a cost of £14 per m^2, 85-fold less than the flat sheet membranes mentioned above.

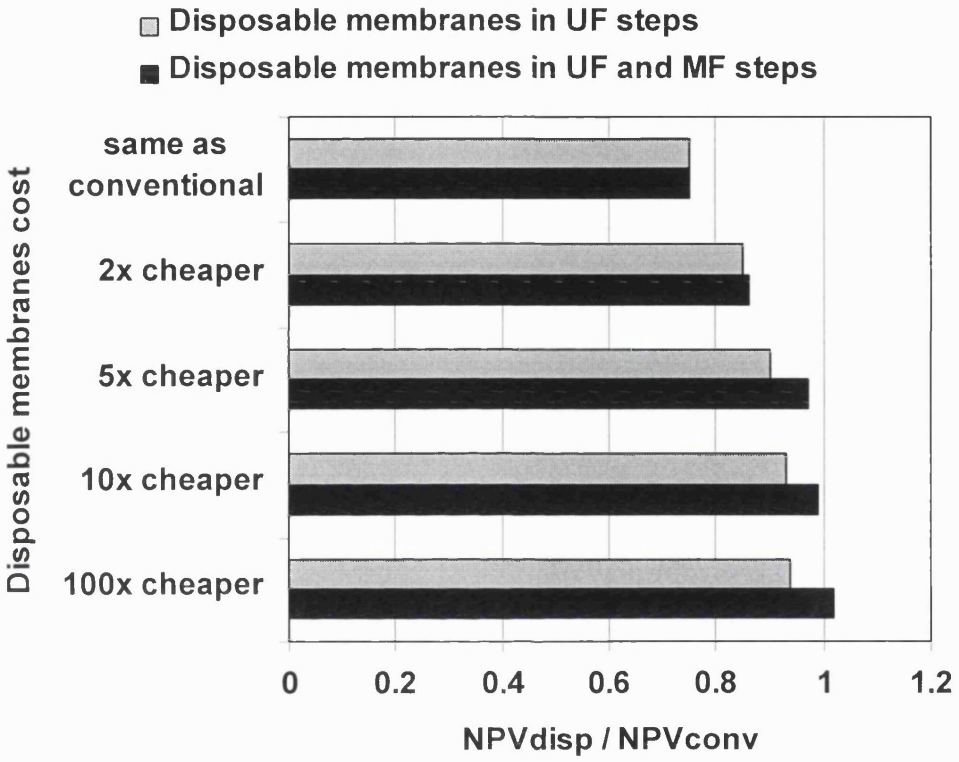


Figure 4.9 Impact on the NPV ratio (NPVdisp/NPVconv) of the use of intrinsically disposable membranes in the UF or/and MF steps of the disposables-based process.

The second set of bars in Figure 4.9 considers that additionally to the UF membranes, the MF step can also be operated with a disposable membrane. A distinction between MF and UF was made, as it may be more difficult to find a disposable MF design that will cope with the high cell concentrations that arise in the cell-harvesting step. On the assumption that both are technically feasible, a 10-fold reduction in the membranes costs is then sufficient to make the disposable option economically identical to the stainless steel option.

The decrease in materials costs may also happen through the use of cheaper (and possibly less robust) matrices in the ion exchange step of the disposables-based

process. Data from Table 4.3 shows that, even though DEAE Cellulose (Whatman) costs 8 times less than DEAE Sepharose FF (Amersham Pharmacia Biotech), which is an agarose matrix cross-linked for improved thermal, chemical and organic solvent stability, this has only a limited impact on the NPV ratio (Figure 4.10).

Matrix (Manufacturer)	Price	pH range	Capacity	Price/capacity
DE52 - DEAE Cellulose (Whatman)	£0.31 per g matrix	2-9.5	700 mg BSA per g	£0.44/mg BSA
DEAE Sepharose FF (Amersham Pharmacia Biotech)	£0.39 per mL matrix	3-12 (long term) 1-14 (short term)	110 mg HSA per mL	£3.55/mg HSA

Table 4.3 Properties and price of two different ion exchange matrices (Whatman catalogue, 2001; Amersham Pharmacia Biotech catalogue, 2000).

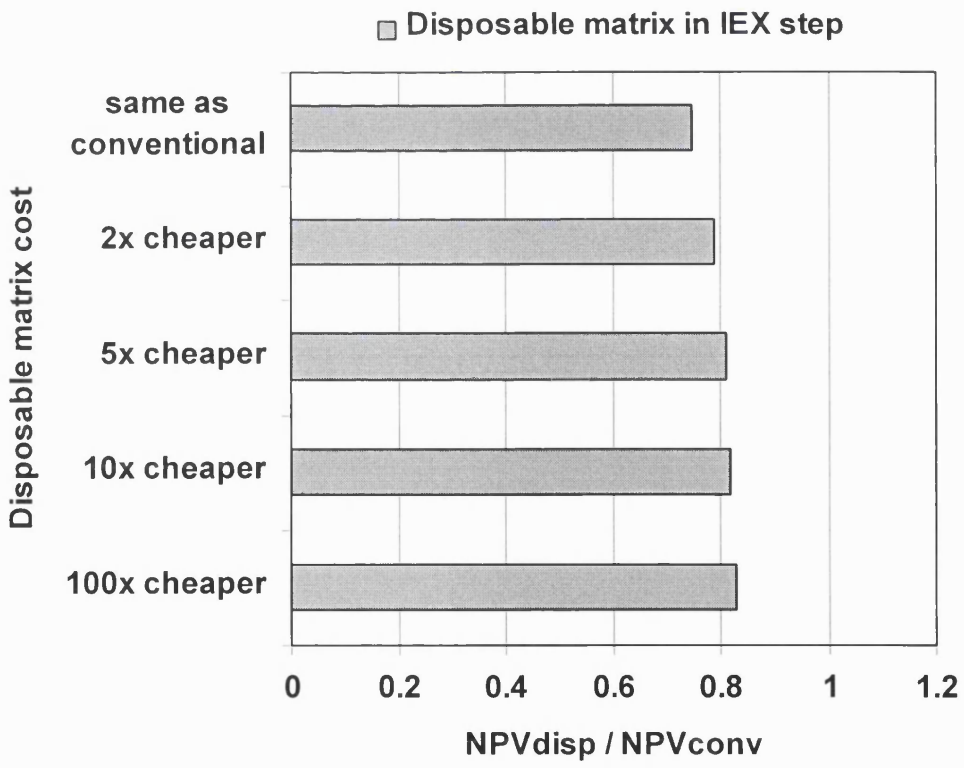


Figure 4.10 Impact on the NPV ratio (NPVdisp/NPVconv) of the use of intrinsically disposable matrices in the IEX chromatography step of the disposables-based process.

4.4.4 Yield

Although the disposables-based process is intended to be identical in performance and characteristics to its conventional counterpart it may well be that some unit operations have to be altered in order to be operated in a fully disposable fashion. Such changes may affect negatively the yield of these particular steps and consequently either more time has to be spent in process development or the fermentation volumes may have to be increased so that the final product mass is the same as in the conventional alternative. This will also impact the design specifications of subsequent operations. For example, the membrane area needed for cell harvesting has to be increased to cope with a higher fermentation volume. The ultimate effect of such differences is a lowered NPV for the disposable plant, which has to be quantified and compared to that of the conventional plant. The evaluation of the impact of a change of yield on the overall process is a complex one with each stage having a different impact on the overall process according to product location and subsequent recovery and purification stages. Hence the impact of the performance of different stages has to be assessed specifically for that stage.

The unit operations that may be affected by a switch to disposables operation are the fermentation, where a stirred tank has to be replaced by a plunging jet (Zaidi, et al., 1991) or an airlift reactor, and the chromatography step, where the column may be replaced by a column pre-packed with cheaper media or by multiple batch adsorption/desorption steps. The microfiltration, ultrafiltration and periplasmic release steps are not expected to be affected as they remain intrinsically the same as in the conventional process.

The performance of a disposable fermenter may be lower when compared to a conventional fermenter as a result of different factors such as oxygen transfer difficulties. Work carried out in this research group (Baker, 2001) with a disposable fermentation plunging jet design indicated that oxygen transfer might be a factor affecting the yield. The yield loss may be due to achieving a lower level of biomass or through the cells being intrinsically less productive (lower expression levels). Additionally, in the particular case of a periplasmically expressed product some material may be released into the fermentation broth during the operation of a disposable fermentation as is observed in stirred-tank fermenters (Gill, et al., 1998).

The effect on the disposable plant NPV of a reduction of 25% in the yield of antibody fragment was studied. It was considered that this reduction in yield could be effected in two different ways: (a) as a 25% reduction in the biomass and (b) as a 25% fall in the expression level of the cells, but with an overall identical biomass concentration (Figure 4.11). Alternative (b) also considers the case where there is a reduction in the amount of product found in the periplasmic space with an equivalent increase in that found in the extracellular medium. The NPV of each alternative was calculated as described in section 4.3.3 considering that the final product mass obtained had to be the same as in the base case. A 25% yield loss in terms of biomass results in a slight drop in the achievable NPV to 91% of that of the base case, whereas a 25% lower expression level has a higher impact on the NPV, decreasing it to 83% of that of the base case (Figure 4.11).

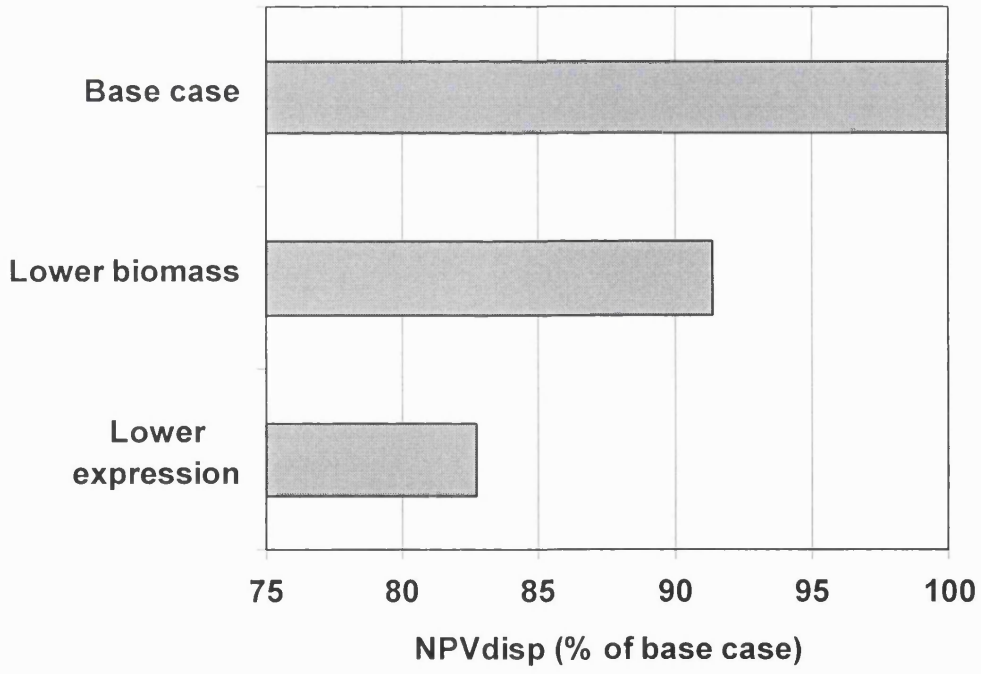
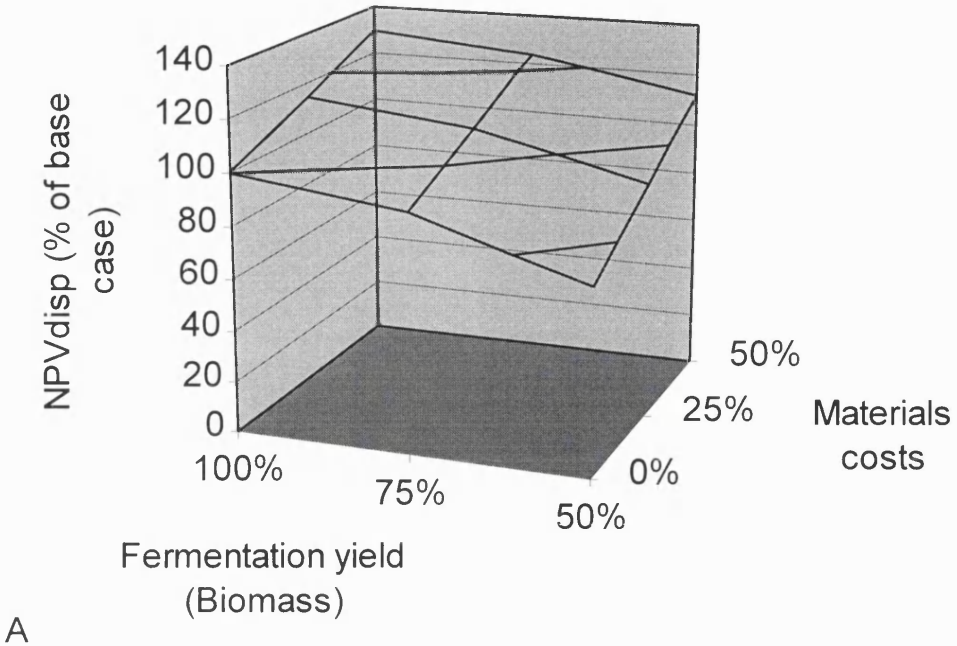


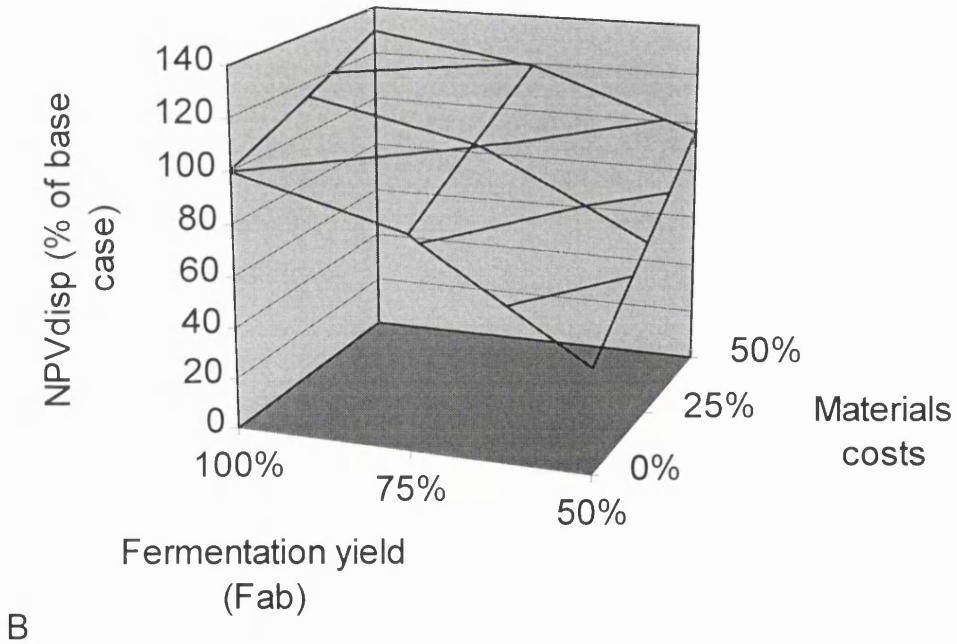
Figure 4.11 Impact of lower fermentation performance effects on the NPV of the disposable option (Novais et al., 2001). It was considered that the reduction in yield could be effected in two different ways: as a 25% reduction in the biomass (Lower biomass) or as a 25% fall in the expression level of the cells, but with an overall same biomass concentration (Lower expression). The results are presented as % of the NPV of the base case, i.e a disposables-based plant with same yield as a conventional plant.

Each of these effects may also be examined more closely. Sensitivity analysis was carried out for a range of fermentation yields of 50% to 100% of the conventional base case, combined with sensitivity analysis for the cost of the materials (raw materials and disposable equipment) and is shown in Figure 4.12A and B. Figure 4.12A illustrates the case where the yield loss is associated with a reduction in biomass whereas Figure 4.12B analyses the consequences of yield loss due to less product being produced by the cells. A 50% drop in biomass yield results in a 30% drop in the NPV. This is more accentuated in the case where the yield loss arises from a lower expression level, resulting in a 60% decrease of the NPV. The cost of the materials was examined as the estimate in the case study was considered to be an upper limit (section 4.4.3). In both cases a 50% saving in materials costs compensates for the loss in yield, bringing the NPV up to 112% (Figure 4.12A) and 96% (Figure 4.12B) of that of the base case.

The final source of process yield variation considered in this study was that due to a lower yield in the disposables chromatography format. This may be thought to arise as a consequence of less specific binding resulting in product loss in the wash step. Alternatively a reduced yield might also result due to a lower capacity of the matrix for the product. The first case requires the use overall of larger process volumes while the second case results in the need for higher volumes of matrix and of chromatography buffers. The second scenario was analysed here. Figure 4.13 shows the results of a sensitivity analysis performed for a reduction in the chromatography yield in combination with a reduction in the cost of the materials. This is a very likely case since the choice of a matrix with a lower capacity would most certainly be driven only by economic considerations. The NPV decrease is small at only 10% when the chromatography yield is 50% lower and this can be compensated for by a 25% saving in materials costs, for which case the NPV would be 107% of that of the base case.



A



B

Figure 4.12 Combined sensitivity analysis on the disposable plant NPV for fermentation yield and cost of materials (Novais et al., 2001). The fermentation yield was varied from 50% to 100% of the yield obtainable with the conventional plant. The reduction in the fermentation yield was considered to be a result of A) lower biomass obtainable and B) lower expression level of the cells. A reduction in the cost of the materials from 0 to 50% allowed the impact of these costs on the NPV of the disposable plant to be investigated.

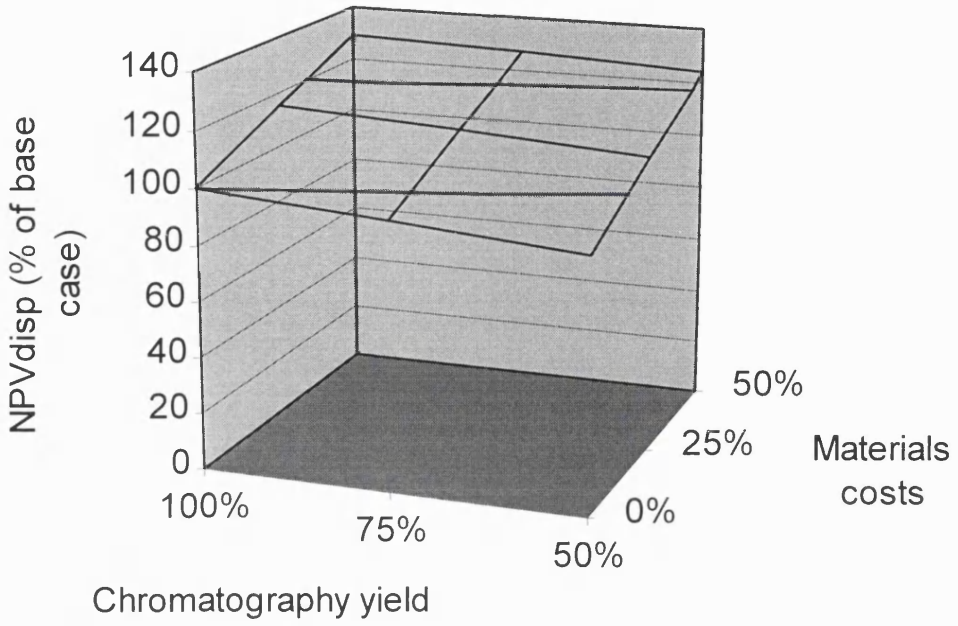


Figure 4.13 Combined sensitivity analysis on the disposable plant NPV for chromatography yield and cost of materials. The yield of the chromatography step was varied from 50% to 100% of the yield obtainable with a conventional chromatography column. Reduction in yield was considered to be a result of a lower capacity of the disposable matrix for the product. A reduction in the cost of the materials from 0 to 50% allowed the impact of these costs on the NPV of the disposable plant to be investigated.

4.5 Discussion and conclusions

The objective of this chapter was the economic comparison of a conventional bioprocessing plant based on stainless steel equipment with one based on disposable equipment. Although the NPV values indicate the conventional option to be the most attractive ($76M for disposables and $103M for conventional), the difference at only 25% is probably sufficiently close to make disposables a viable alternative, especially when considering the other advantages of disposable plants outlined in the introduction, e.g. time savings and flexibility.

The lower capital investment of the disposable option results in increased flexibility for the disposable plants since changes in the process or the product result in a reduced financial loss, which is of great interest for start-up companies. It also allows for an earlier decision to build which may result in earlier entry to market. This effect benefits strongly the disposables option since a reduction in time to market has a high impact on the NPV ratio, as shown in the results of the sensitivity analysis (Figure 4.8).

Turning now to consider the differences between the two modes of processing it is clear that staff costs have only a marginal effect on the way the conventional and the disposable options compare. This means that even if the staff requirements of a disposable plant are less than those of a conventional plant that would only result in a slight increase of the NPV ratio. Similarly a decrease of 25% in the capital investment would result in a variation of less than 5% on the NPV ratio, showing insensitivity to this variable. Materials costs are shown to be more influential leading to the need for a more detailed evaluation of the impact of this variable on the NPV ratio. Indeed the examples given in section 4.4.3 show that materials costs may be reduced by even more than 25%, in favour of the disposable option. For example an overall 10 fold decrease in the cost of membranes is probably an attainable target and results in identical NPVs for both options. This cost reduction could be achieved by making use of disposable dialysis cartridges. Also the cost of membranes can be expected to decrease once a market for disposable equipment has been established and economies of scale develop, as in the medical device market.

A final difficulty encountered in the analysis of disposable bioprocessing is to establish the degree of similarity between disposables-based plants and conventional designs. The fermentation step is an example of how the different engineering features of a disposable plant could have a detrimental impact on product yield. Although the impact on yield may happen in different ways, an analysis of the sensitivity in Figure 4.11 shows that this is expected to affect the obtainable NPV by less than 20%.

Figure 4.12A and Figure 4.13 show that the reduction in the achievable yield given by a disposables-based option and associated both with a lower level of biomass production in the fermentation and a reduced chromatographic performance has only a

limited impact on the NPV that is realised. By contrast the loss of yield due to less productive cells has a dramatic impact on the NPV (Figure 4.12B). This is however a less likely scenario.

The analysis shows that any fall in NPV can easily be overcome when producers of disposable equipment start responding to an increasing demand in their products with higher production scales and hence lower prices. Effectively, a 50% reduction in the yield of the fermentation can be compensated for by a 50% reduction in the cost of the materials (Figure 4.12A and B). This is even more striking in the case of the chromatography (Figure 4.13) where even a 50% loss in yield can be overcome by a saving of 25% in the materials costs; a margin that appears highly probable as the disposables approach starts to gain acceptance.

It has to be noted that the use of a chemical engineering model for the calculation of the running costs indicated disposable plants as the more attractive option (section 4.3.2), with lower operating costs and consequently a higher NPV. This result is contrary to what could be intuitively predicted from the definition of a disposables-based plant where the increase in the variable costs associated with disposable equipment would be expected to have a higher impact on the NPV. This is because this model places more emphasis on costs such as depreciation and utilities, which are reduced in a disposables approach, rather than on raw materials and consumables. The biochemical engineering model for running costs presented in section 4.3.2 was considered more appropriate and was therefore used for the NPV calculations. This does, however, show how critical it is to identify an appropriate running costs model, a common problem found in biotech costing studies.

In conclusion, a disposables-based plant with the same features as its conventional equivalent is economically and conceptually attractive as it may be of easier and quicker implementation and with a comparable overall investment required. Not only does it present a NPV which is close to that of a conventional option, the difference can actually be nullified with the use of intrinsically disposable equipment or by achieving shorter times to market.

The high impact of the cost of membranes sets the scene for the next chapters of this thesis. Part II will start with a Materials and methods chapter followed by a study of

the mechanism of performance decay in the lysate clarification step of the case study process. Chapters 7 and 8 will then focus on experimental strategies to reduce the membrane area required in biopharmaceutical processes.

PART II

ENGINEERING ASPECTS OF DISPOSABLES-BASED BIOPROCESSING

Chapter 5 Materials and methods

5.1 Materials

All reagents were from BDH chemicals (Merck Ltd., Lutterworth, Leicestershire, UK) unless otherwise stated and were of the highest grade available.

5.1.1 Process material

5.1.1.1 Bacterial strain and plasmid

The strain used in the fermentation was a wild type *E. coli* W3110 (ATCC 27325) transformed with the plasmid pAGP-4. The plasmid pAGP-4 encoded the chloramphenicol resistance gene (Cm) and the 4D5 Fab' antibody fragment directed against the extracellular domain of $p185^{HER2}$ and derived from HumAb4D5 (Carter et al., 1992, Kelley et al., 1992). Coding sequences for the Fab' light chain and heavy chain Fd' fragment were arranged in a dicistronic operon under transcriptional control of the *E. coli tac* promoter inducible by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) or lactose. Each antibody chain was preceded by the *E. coli omp A* signal sequence to direct secretion to the periplasmic space.

5.1.1.2 Fermentation and cell harvesting

A single 450 L fermentation was carried out with defined medium with the following composition (gL^{-1}): $(NH_4)_2SO_4$, 5; NaH_2PO_4, 2.88; KCl, 3.87; $MgSO_4.7H_2O$, 0.717; citric acid, 4; trace elements, $1mLL^{-1}$; PPG (25% v/v), 1 mLL^{-1}; glycerol, 3% (w/v); cloramphenicol, 30 μgmL^{-1}. The trace elements solution was composed of (gL^{-1}): citric acid, 10; $CaCl_2.6H_2O$, 0.5; $ZnSO_4.7H_2O$, 0.246; $MnSO_4.4H_2O$, 0.2; $CuSO_4.5H_2O$, 0.05; $CoSO_4.7H_2O$, 0.0427; $FeCl_3.6H_2O$, 0.967; H_3BO_3, 0.003; $NaMoO_4$, 0.0024. The growth temperature was reduced from 30 to 27°C at an OD_{600} of 40. The following additions were also made: 30 gL^{-1} glycerol (15 OD), 20 gL^{-1} glycerol (35 OD), $MgSO_4.7H_2O$, 14.4 mM final concentration and $CaCl_2.6H_2O$, 1.7 mM final concentration (40 OD), 10 gL^{-1} glycerol and 45 gL^{-1} lactose (50 OD). The pH was maintained at 6.95. More details of the fermentation protocol have been described elsewhere (Bowering, 2000). The fermentation provided the material used in all microfiltration experiments.

The cells were harvested from the fermentation broth with a Sharples centrifuge, model AS26 (Alfa-Laval Engineering Ltd, Camberly, UK) at 17 000 rpm (19 000 g) and at an operating flow rate of 60 Lhr^{-1}. The cell paste was then stored at –70°C in 1 kg aliquots.

5.1.2 Buffers

5.1.2.1 Periplasmic extraction buffer

A periplasmic extraction buffer was prepared by dissolving pre-weighed quantities of Tris[hydroxymethyl]aminomethane (Trizma Base, Sigma) and Ethylenediaminetetra-acetic acid (EDTA, Sigma) in water purified by reverse osmosis (RO water, 20-60 μScm^{-1}) to a final concentration of 100 mM and 10 mM respectively. The pH was adjusted to 7.4 with HCl or H_3PO_4.

5.1.2.2 Diafiltration buffers

The diafiltration buffer was prepared by dissolving a pre-weighed amount of sodium chloride (NaCl, Sigma) in RO water to a final concentration of 150 mM.

NaCl prepared to a final concentration of 100 mM was also used as diafiltration buffer in one experiment.

5.1.2.3 Chromatography buffers and reagents

The buffers and reagents used in the Fab' purification step include 6M guanidine HCl, 20% ethanol, 50% (w/v) sodium glycinate and 2 M Tris.HCl, pH 8.5, all prepared in ultra pure water with a resistivity greater than 18 MΩcm obtained from a Elgastat Maxima – HPLC water purification system (Elga Ltd, High Wycombe, UK).

The equilibration buffer, Buffer A, is 1 M glycine, pH 8.0, obtained by dissolving 150g of glycine (Sigma) and 4.5 g of sodium glycinate in 2 L of ultra pure water. The elution buffer, Buffer B, is 0.1M tri-sodium citrate/citric acid at pH 3.0.

5.1.2.4 ELISA Buffers

Phosphate buffered saline (PBS) was prepared by dissolving 8 g of NaCl, 0.2 g of KCl, 1.15 g of Na_2HPO_4 and 0.2 g of KH_2PO_4 in 1 L of ultra pure water. The final pH was 7.1-7.3. PBS/Tween was PBS to which Tween-20 was added to a final concentration

of 0.05% (v/v) or obtained by dissolving the contents of a PBS/Tween sachet (Sigma) into 1 L of ultra pure water.

Sample conjugate buffer was prepared by dissolving 6.05g of tris amino-methane, 2.92 g of NaCl, 1 g of casein and 0.1 mL of Tween-20. The pH was adjusted to 7.0 with HCl and the solution was filtered with a paper filter before storage.

The substrate solution was prepared immediately before use through the addition of 100 μL TMB (Tetramethylbenzidine, Sigma, made up as a 10 mg L-1 solution in dimethylsulphoxide, Sigma, and stored in the dark) and 100 μL of H_2O_2 (1 in 50 solution of 30% H_2O_2, Sigma, in ultra pure water) to 10 mL of acetate buffer (0.1 M sodium acetate/citric buffer pH 6.0).

5.1.3 Periplasmic release (lysate preparation)

Frozen *E. coli* cell paste was resuspended in pre-heated (40°C) 2 to 2.5 L of periplasmic extraction buffer to a final concentration of 283 g of cells (wet weight) per 2 L of buffer, i.e. 47 g dcw L^{-1}. After complete suspension the mixture was heated to 60°C for 3 hours in a LH Series 210 fermenter (LH Fermentation, Inceltech UK Ltd., Berkshire, UK) stirred at 300 rpm. The resulting spheroplasts suspension was then cooled down to room temperature and used for filtration experiments or stored at 4°C for up to three days if not used the same day. In the present work the term lysate will refer to this suspension of spheroplasts.

5.2 Filtration

5.2.1 Filtration equipment

The membrane filtration experimental set-up used was a ProFlux® M12 rig (Millipore Corporation, Bedford, MA, USA) equipped with two Masterflex® L/S™ (Cole-Parmer Instrument Company, Vernon Hills, IL, USA) peristaltic pumps, one for retentate recirculation (Easy-Load® II Model 77201-62) and a second pump to control permeate flux (Quick Load® Model 70201-24) (see Figure 5.1). The system also includes three pressure transducers to measure inlet, outlet and permeate pressures connected to a digital display.

The membrane used for lysate clarification was a 0.1 m^2 polyethersulfone (BIOMAX) cassette membrane (Pellicon 2 Mini) from Millipore, fitted into a stainless steel Mini Cassette holder. The membrane had a molecular weight cut-off (MWCO) of 1000 kDa and open channels with turbulence screens designed to minimise deposition of fouling (v-screen).

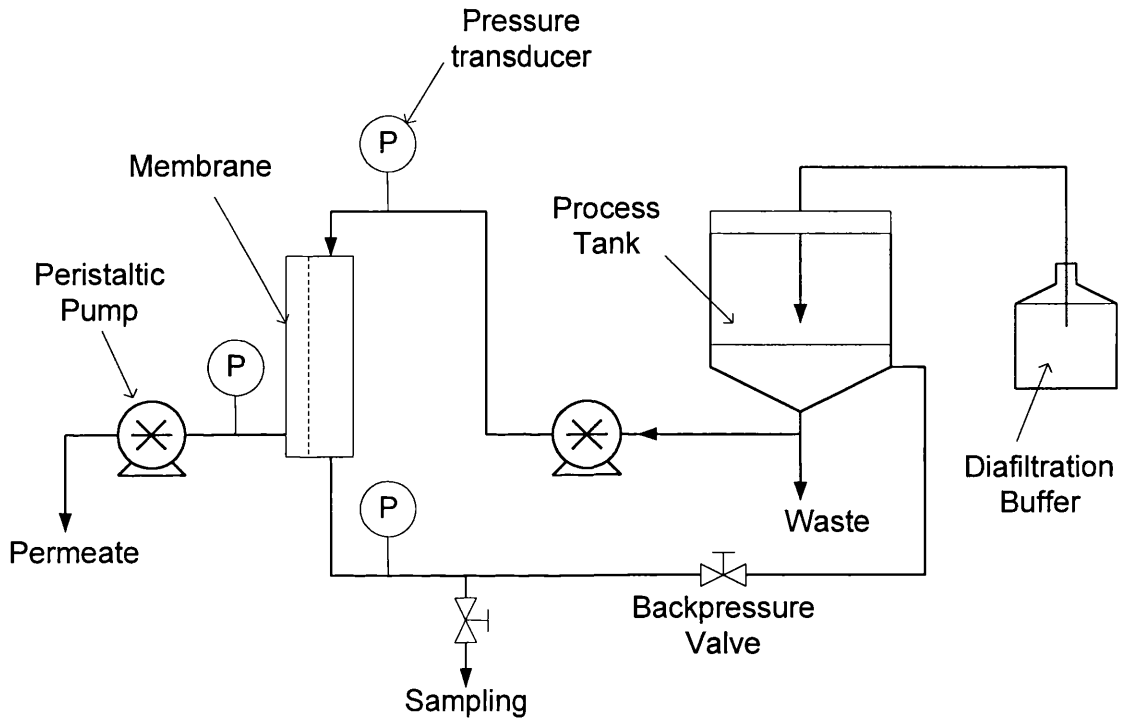


Figure 5.1 Diagram of the experimental set-up.

Two polyethersulfone UF membranes of 10 kD MWCO (Pellicon 2 Mini, Millipore(UK) Ltd, Hertfordshire, UK) and 0.1 m^2 membrane area each were used for the concentration of permeate.

A LabScale™ TFF System (Millipore Corporation, Bedford, MA, USA) was also used for UF concentration of small volumes (<250 mL), mounted with two 50 cm^2 Pellicon XL 10 kD MWCO membranes (polyethersulfone).

5.2.2 Membrane cleaning

Between experiments the membrane system was cleaned with 250 ppm HOCl or with 0.1M H_3PO_4 at 45°C for 20 minutes. With the 1000 kD membrane the feed pump was set at 40% (1.3 L min^{-1}) speed and the permeate pump set at 5% (23 mL min^{-1}) speed. In the case of the two 10 kD membranes the feed pump was set at 25% (765 mL min^{-1}) and no permeate pump was used. The system was then rinsed thoroughly with RO water and the membranes were stored at 4°C in 0.1 M H_3PO_4. The cleaning procedure followed for the Pellicon XL membranes with the LabScale TFF system was similar to the one described above, with no permeate flux restriction.

Pure water flux was measured at different feed flows after cleaning to assess the effectiveness of the cleaning step. When only low values of water flux could be obtained (<50 $mLmin^{-1}$ at 570 $mLmin^{-1}$ recirculation rate and 0.07 bar TMP) the cleaning procedure was repeated.

5.2.3 Determination of critical flux

To find the optimal operating conditions for the system, flux was raised by step increments every 15 minutes and transmembrane pressure (TMP) was monitored. Permeate was recirculated back into the tank to maintain a constant concentration. Permeate samples were taken after flux stabilisation in order to assess the percentage of transmission, defined as the ratio of antibody fragment concentration in the permeate to that in the retentate.

5.2.4 Total permeate recycle experiments

Buffer conditioning with 250 mL of diafiltration buffer was performed before ramped addition to the process tank of 0.5 L of lysate. With the permeate pump off, backpressure valve closed and sampling valve open the buffer flows out of the system. When the lysate approaches the sampling valve, this valve is closed rapidly and the backpressure valve is opened simultaneously. Buffer conditioning is necessary to avoid air pockets in the permeate side, which may have a detrimental effect on transmission (Meagher et al., 1994). The speed of the feed pump was increased to 40% (1.3 $Lmin^{-1}$) and the backpressure valve was closed partially in order to establish an inlet pressure of 0.83 bar. At this point (taken as t=0) the permeate pump was started at 11% (65 mL/min) and the transmembrane pressure (TMP) was set at approximately 0.14 bar

with the backpressure valve. The permeate was continuously fed back into the process tank. The average velocity over the membrane was calculated to be 0.4 m s^{-1} from the recirculation flow and from the internal dimensions of the membrane (Figure 5.2).

Samples were taken from the retentate and from the permeate at regular intervals. The inlet, outlet and permeate pressures were monitored throughout the process.

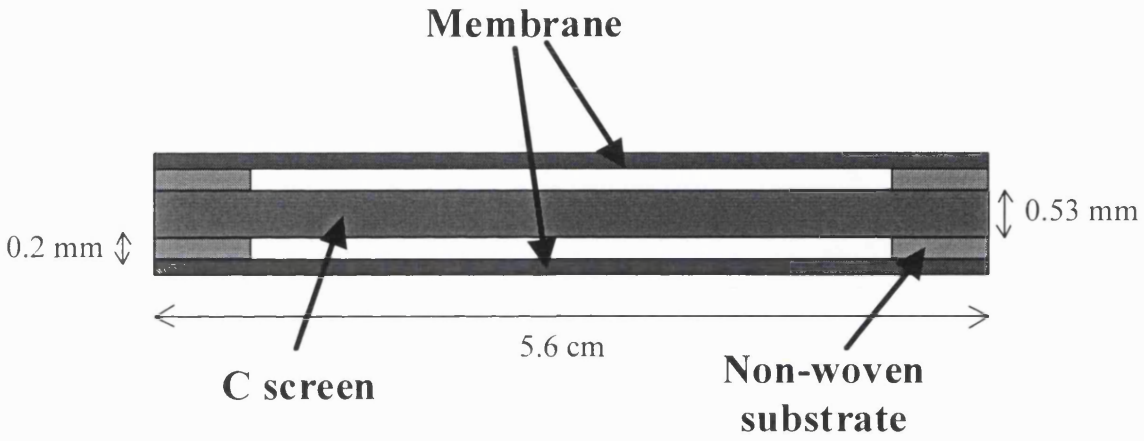


Figure 5.2 Cross section of the feed channel of a Pellicon 2 V-screen mini-cassette (adapted from Christy, 1999 and Millipore Data Sheet, 1998b).

5.2.5 Diafiltration experiments

Buffer conditioning with 250 mL of diafiltration buffer was performed before ramped addition to the process tank of 0.5 L of lysate. The speed of the feed pump was increased to 40% (1.3 $Lmin^{-1}$) and the backpressure valve was closed partially in order to establish an inlet pressure of 0.83 bar. At this point (taken as t=0) the permeate pump was started at 11% (65 mL/min) and the transmembrane pressure (TMP) was set at 0.14 bar. The permeate was collected separately. The permeation starts creating a vacuum in the tank, from which results constant volume diafiltration after 2-3 minutes. The permeate was collected in a separate container and the flux was measured at regular intervals with a measuring cylinder and a stopwatch. The inlet, outlet and permeate pressures were monitored throughout the process.

Samples were taken at regular intervals from the permeate and from the retentate (through the sampling valve in the recirculation loop, see Figure 5.1).

5.2.6 Rinsing experiments

For the rinsing experiments samples were taken from the retentate and from the permeate at t=3, 10, 15 and 20 min. After 20 minutes of diafiltration the process was interrupted through the halting of the feed and permeate pumps simultaneously. The system was drained of the lysate and rinsed with 250 mL of diafiltration buffer which was recirculated for 10 min (no permeate flow). The process was then resumed for another 20 min with a fresh load of lysate or partially processed lysate (from the first 20 min) or even partially processed lysate to which 19 mL concentrated permeate was added (prepared according to the procedure described in 5.2.7 below).

In a different set of experiments the diafiltration buffer was recirculated for only 1 min and the process resumed with either fresh lysate or partially processed lysate to which was added 24 mL of purified Fab' antibody fragment (at a concentration of 800 mgL^{-1}) or 25 mL of concentrated permeate without Fab' (see 5.2.8 below).

5.2.7 Concentrated permeate preparation

The permeate obtained from a diafiltration experiment was collected and stored overnight at 4°C. The initial volume of 1310 mL of permeate was concentrated down to 240 mL with two 10 kD membranes mounted into the the Proflux® M12 rig (total area 0.2 m^2). The process was run in TMP control mode, i.e. without a permeate pump. TMP was maintained at 0.7 bar and the permeate flow decreased from an initial value of 86 mL min^{-1} down to 42 mL min^{-1} in 20 minutes. Constant volume diafiltration was started at this stage for another 15 minutes, which corresponded to approximately 2.5 diafiltration volumes. The partially concentrated permeate was again stored overnight at 4°C. Further concentration was achieved with the aid of a Labscale TFF system and two 10 kD membranes (total area 100 cm^2). TMP was maintained between 1.17 and 1.38 bar and the flux decreased from an initial value of 9 mL min^{-1} down to 6.5 mL min^{-1} after 29 minutes processing. The final volume of concentrated permeate was 19 mL.

5.2.8 Purified Fab' antibody fragment preparation

Purified Fab' was obtained from concentrated permeate by packed bed affinity protein A chromatography using a BioCAD™ 700E workstation (Perceptive Biosystems, Warrington, UK).

A volume of 1240 mL of permeate was concentrated down to 143 mL with two 10 kD membranes mounted into the the Proflux® M12 rig as described above (5.2.7). The process was run in TMP control mode, i.e. without a permeate pump, at 0.85 bar. The concentration process took 23 minutes and the resulting concentrated permeate was stored overnight at 4°C.

A HR 10/10 column (Amersham Pharmacia Biotech, Uppsala, Sweden) of dimensions 10 mm (internal diameter) and 10 cm (bed height) packed with Streamline rProtein A matrix (7 mL) was used. The column was first washed with 1 column volume (CV) of 6M guanidine HCl and equilibrated with buffer A. Solid glycine was added to the concentrated permeate to a final concentration of 1 M and the pH was adjusted to 7.5 with 50% (w/v) sodium glycinate. The sample was fed at 3 mL min^{-1} and the load fraction (138 mL) was collected separately. The column was then washed for 30 minutes with Buffer A. The bound material was eluted with Buffer B until A280 returned to baseline (24 mL), the pH of this Fab' containing fraction was adjusted to 5.5 with 2M Tris.HCl, pH 8.5 and stored at 4°C overnight. The column was washed with 15 mL of GuHCl and 3 CV of ethanol.

The column load was concentrated down to 50 mL with the aid of a Labscale TFF system and two 10 kD membranes, at which point diafiltration was started. TMP was maintained at 1.17 bar. After three diafiltration volumes the feed was further concentrated to a final volume of 25 mL.

5.2.9 Spun-down lysate preparation

E. coli lysate was spun down in a Beckman J2-MI centrifuge (Spinco, Beckman Instruments, CA, USA) with a JA10 rotor for 45 minutes at 10,000 rpm, 4°C. The supernatant was collected and used as the feed for a microfiltration experiment.

5.3 Analytical techniques

5.3.1 Sample preparation

All retentate and permeate samples were stored at –20°C and thawed at room temperature prior to centrifugation at 13 000 rpm (~14 000 g) for 10 minutes in a Microfuge™ 11 (Beckman, CA, USA). The supernatant was used for the analytical procedures.

The impact of overnight freezing vs. overnight storage at 4°C was assessed for the lysate. The frozen sample did show a higher quantity of Fab' fragment, but the difference between the two measurements was 8%, less than the error of the ELISA assay (11%, Bowering, 2000) and hence not deemed significant.

5.3.2 Total protein assay

Total protein concentration was determined by the Bradford assay technique using Coomassie Brilliant Blue G-250 reagent (Bio-Rad Laboratories Ltd., Hertfordshire, UK). Bovine Serum Albumin (BSA) protein (Pierce and Warriner (UK) Ltd, Chester, UK) was used as the standard, prepared in dilutions in the range 0.2-1.0 $mgmL^{-1}$. Samples to be assayed were also diluted to within the same range with PBS buffer.

50 µL of sample and 2.5 mL of assay reagent were mixed in a cuvette and the change in absorbance at 595 nm was recorded after 5 minutes in a DU-® Spectrophotometer, (Beckman Instruments (UK) Ltd., High Wycombe, UK). Protein concentrations of the samples were determined from a calibration curve of A_{595} vs. BSA concentration.

For very diluted samples (≤25 μgmL^{-1}) a microassay procedure was followed, for which a range of dilutions of 5-25 μgmL^{-1} of the standard where used. In this case 0.5 mL of sample or standard were mixed with 0.5 mL of assay reagent for protein concentration determination.

The Bradford protein assay allows determination of protein concentration to within +/- 5%.

5.3.3 ELISA

ELISA was used as a means to quantify Fab' antibody fragment. NUNC 96 well maxisorp immunoplates (Life Technologies Ltd, Paisley, UK) were coated overnight at 4°C with HP6045 (a mouse antihuman monoclonal antibody supplied by Celltech Chiroscience Ltd) at a concentration of 2 μg mL^{-1} in PBS. After 4 washes with PBS/Tween in a Columbus Washer (Tecan UK Ltd, Reading, UK), purified Fab' standard (2 lanes) and samples (remaining lanes) appropriately diluted in sample conjugate buffer were added to the top row of the plate. A serial of 1 in 2 dilutions was performed on the plate in 100 μL of sample conjugate buffer and the plate was placed on a 3D rocking platform STR9 (Suart Scientific, UK) at room temperature for 1 hour at 30 rpm. The wash step was repeated and 100 μL of GD12 peroxidase (The Binding Site Ltd) was added to each well in a dilution of 1 in 2000 of sample conjugate buffer. The plate was again incubated on a rocking platform in the same conditions as before for 1 hour. A further washing step was carried out and 100 μL of the substrate solution was added to each well. The absorbance at 630 nm was recorded with a Titertek Multiskan® PLUS MK II microplate reader (Flow Laboratories, High Wycombe, UK) after 5-7 minutes. The concentration of Fab' was determined from a standard curve prepared for each plate.

5.4 Transmission

The transmission of the Fab' antibody fragment (or of total protein) through the membrane was calculated from the following expression:

$$\%\text{Transmission} = \frac{Cp}{Cr}$$

Equation 5.1

where Cp and Cr are the concentration of Fab' (or total protein) in the permeate and retentate respectively. Cp and Cr were evaluated by the analysis of the collected samples through ELISA (or the Bradford assay).

The methods described in this chapter provided the experimental protocols for the following chapters.

Chapter 6 Crossflow separation of a Fab' antibody fragment form *Escherichia coli* lysate

6.1 Introduction

The objective of this chapter is to characterise fully the system so that its performance can be optimised, i.e. minimize the required membrane area. This will be a key concern in a disposables-based membrane separation.

The performance of the system can be improved by reducing the extent of fouling occurring during the process. Based on this premise, it was decided to operate at constant permeate flux, thus avoiding overfouling in the initial stage of filtration (Defrance and Jaffrin, 1999a). The first step was therefore to identify the critical flux below which heavy fouling does not occur (section 6.2.1) and to choose an appropriate operating permeate flux.

Experiments evaluating the behaviour of transmission of the antibody fragment through the membrane were then performed, both in total permeate recycle mode of operation (section 6.2.2) and constant volume diafiltration (section 6.2.3). The conclusions made on the tangential flow filtration of *E. coli* lysate for the recovery of an antibody fragment are then presented in section 6.3.

6.2 Results and discussion

6.2.1 Determination of critical flux

The critical flux concept, introduced by Field et al. (1995), indicates that there exists a flux above which fouling is observed. The fouling threshold can be studied with a "stepping" flux experiment (Chen, 1998) and can be detected when the transmembrane pressure drop, TMP, starts increasing at a fixed imposed flux. This indicates that irreversible deposition has begun to occur. The identification of the critical flux is especially important for lysate clarification, as this is an inherently difficult operation (Bailey and Meagher, 2000).

To find the optimal operating conditions for the present system, flux (J) was raised by step increments every 15 minutes and TMP was monitored (Figure 6.1). This time interval was found to be sufficient for TMP stabilisation with other systems (Defrance and Jaffrin, 1999b). Permeate was continuously recirculated back to the tank to maintain a constant concentration of solids and product in the tank. As the flux reached a critical value of 40 $Lm^{-2}h^{-1}$ the TMP was seen to increase suddenly, indicating severe fouling had occurred (Figure 6.2).

Permeate samples were taken after stabilisation at each value of flux in order to assess the percentage of transmission (%T), defined as the ratio of antibody fragment concentration in the permeate to that in the retentate. The increase in TMP was accompanied by a drop in transmission which further indicates fouling had taken place (Figure 6.3). The high range of variation between the different repeats of the experiment may be due to different initial membrane conditions. The lysate itself may also present some variability, with more or less released intracellular contents. Due to the variability between the different experiments it was not clear whether there is an initial increase of %T with increasing flux, before the drop at the critical region. According to the stagnant film model one would expect an initial decrease of %T with increasing flux, followed by an increase towards 100% (Opong and Zydney, 1991) in an ideal, non-fouling environment. The initial decrease of %T may be apparent in the present case for values of flux between 8 and 12 $Lm^{-2}h^{-1}$, although this might also be a result of experimental variation. Meacle et al. (1999) also indicate that an increase in flux can contribute to a rise in transmission due to increased concentration-polarization of the species to be removed. However these authors also report that high fluxes may also result in increased fouling due to cake build-up or compaction or even due to membrane compaction. Effectively in the present case %T rises with increasing flux until it reaches a maximum and then starts decreasing, possibly due to having passed the fouling threshold. According to Chen et al. (1997) such a critical flux corresponds to a transition from concentration polarization to cake formation, or a transition from reversible to irreversible fouling (Defrance and Jaffrin, 1999b). In the present case there may also be some time related decrease of %T due to progressive fouling, as a result of the length of the experiment (over two hours).

The key result in any case is that beyond a critical value of flux of approximately 35-40 $Lm^{-2}h^{-1}$ %T starts decreasing. The maximum in %T also coincides with a maximum in mass flux rate of antibody fragment, defined as the flux rate multiplied by the concentration of antibody fragment in the permeate, $J \times C_p$ (Figure 6.4).

Based on these results the lysate needs to be processed at a controlled flux of less than 40 $Lm^{-2}h^{-1}$ but as high as possible so as to allow for a high productivity just outside the critical region. These conditions also allow for long term operation due to the lower extent of fouling observed below the critical flux (Defrance and Jaffrin, 1999a).

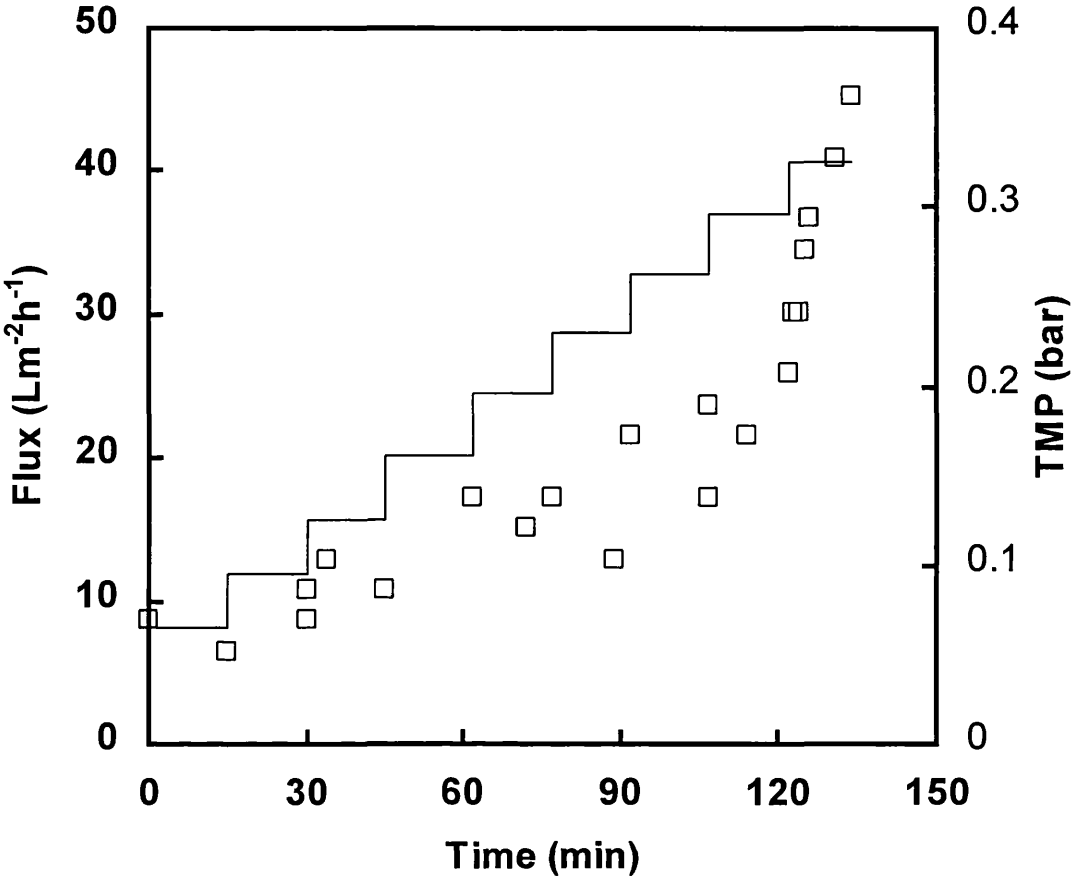


Figure 6.1 Relationship between transmembrane pressure drop (□) and flux rate (—), step-increased at intervals of 15 minutes, and time, shown for only one repeat of the experiment (presented in Figure 6.2 with the same symbol). Experiments conducted under total permeate recycle to maintain constant retentate properties (equivalent cell concentration 47 g dcw L^{-1}) and constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). The volume of lysate was 1 L.

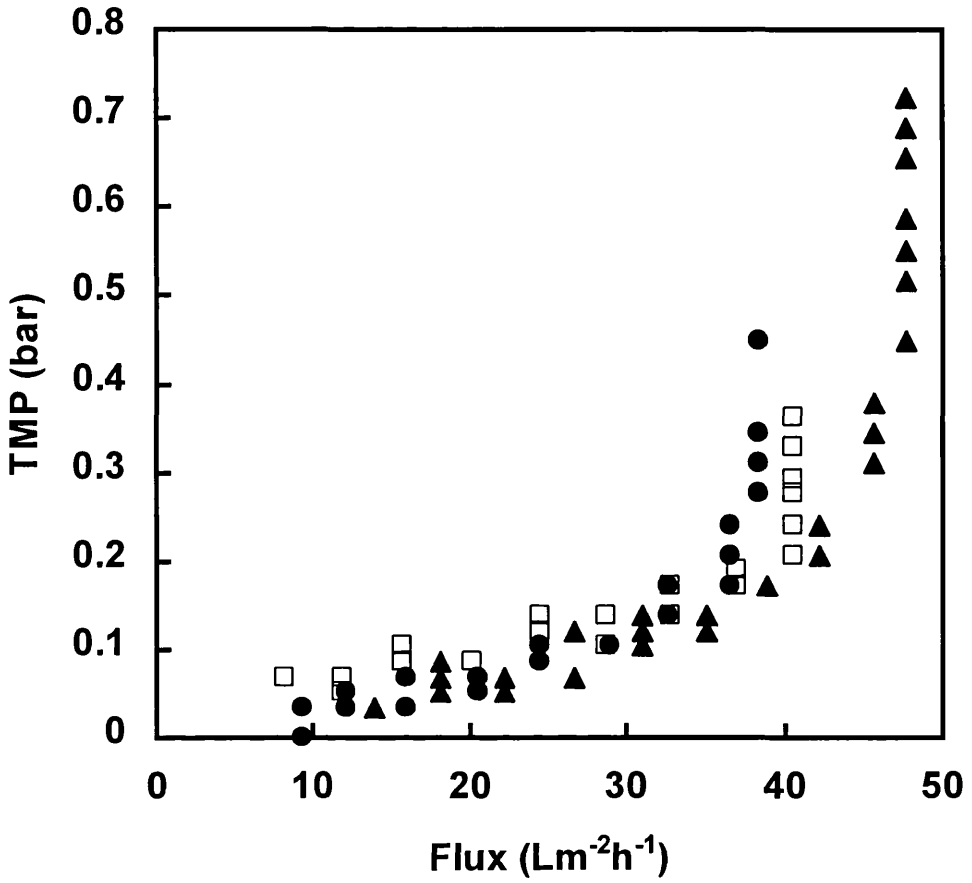


Figure 6.2 Relationship between transmembrane pressure drop and the flux rate, step-increased at intervals of 15 minutes. The three repeats of the experiment were conducted under total permeate recycle to maintain constant retentate properties (equivalent cell concentration 47 g dcw L^{-1}) and under constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). The volume of lysate was 1 L.

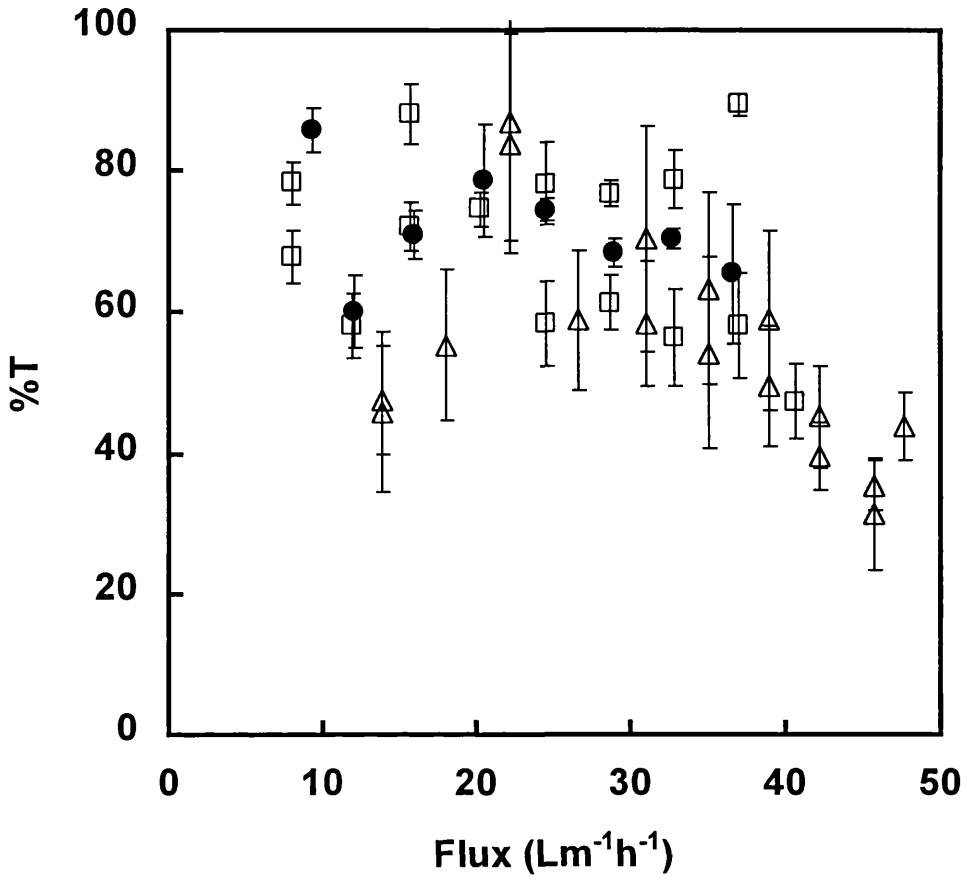


Figure 6.3 Relationship between transmission of antibody fragment through the membrane, i. e. ratio of antibody fragment in permeate and retentate, and flux rate, which was step increased every 15 minutes. The symbols □, ● and △ correspond to three repeats of the experiment. Repeat measurements of the same sample are shown with separate markers. Error bars represent the propagated error from the standard deviation as a result of two to four dilutions of each permeate and retentate sample. Experiments conducted under total permeate recycle to maintain constant retentate properties (equivalent cell concentration 47 g dcw L^{-1}) and constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). The volume of lysate was 1 L.

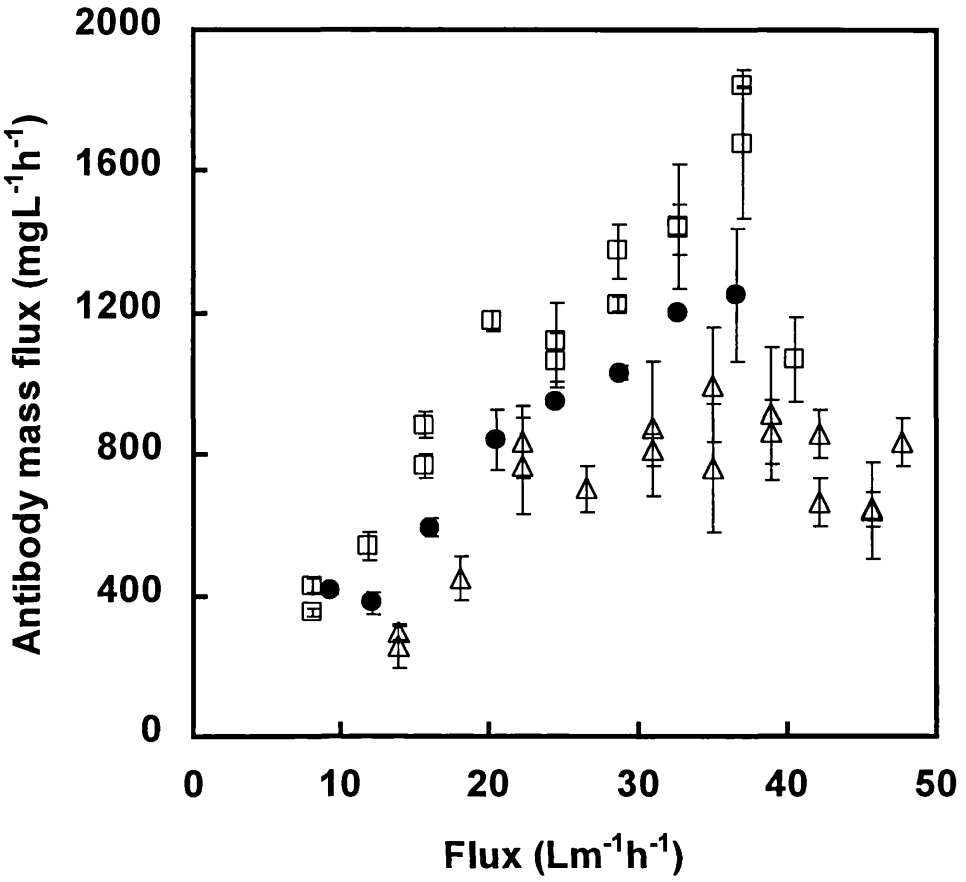


Figure 6.4 Relationship between mass flux of antibody fragment through the membrane, i.e. concentration of permeate multiplied by the flux rate, and flux rate, which was step increased every 15 minutes.. The symbols □, ● and △ correspond to three repeats of the experiment. Repeat measurements of the same sample are shown with separate markers. Error bars represent the propagated error from the standard deviation as a result of two to four dilutions of each permeate sample. Experiments conducted under total permeate recycle to maintain constant retentate properties (equivalent cell concentration 47 g dcw L^{-1}) and constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). The volume of lysate was 1 L.

6.2.2 Total permeate recycle

Figure 6.5 and Figure 6.6 show the relationship between the percentage transmission of antibody fragment with time in experiments where the permeate was recirculated back into the tank (total permeate recycle). This mode of operation is useful for process characterisation since it maintains the same feed conditions throughout the process. Each %T was calculated as the ratio of the corresponding permeate concentration over an average value of the retentate concentration for t=3, 10, 25 and 40 minutes.

For the highest flux (J = 39 $Lm^{-2}h^{-1}$, Figure 6.5) the %T remains high at 80-85% throughout the 40 minutes of the process, indicating that significant fouling is not occurring. There seems to be a tendency for a decrease after 40 minutes of operation but the very low value of r^2 does not allow a firm conclusion to be drawn about the trend.

In the case of a lower flux (J = 19 $Lm^{-2}h^{-1}$, Figure 6.6) the same trend is observed for one of the repeats for the first 40 minutes followed by a stronger drop in transmission to 55-60%. The other repeat for J = 19 $Lm^{-2}h^{-1}$ shows an overall lower transmission, resulting from an earlier and more accentuated decay. This might have been due to a poorly cleaned membrane or a more fouling lysate.

The two permeate fluxes do not present striking operating differences and the higher flux is therefore more appropriate for the separation process, since it corresponds to a much higher productivity as seen from Figure 6.4. In fact it would even seem that operation at 19 $Lm^{-2}h^{-1}$ presents a lower %T, although this would have to be confirmed with further experiments and is beyond the scope of the present work.

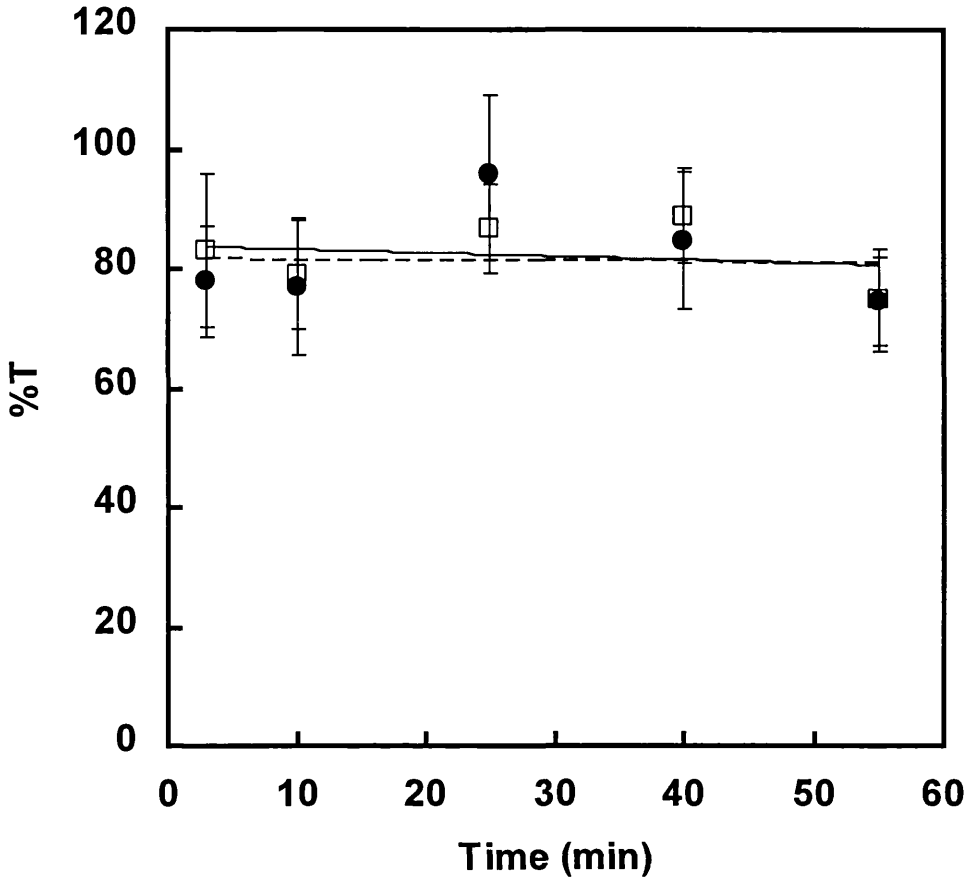


Figure 6.5 Effect of time on the transmission of antibody fragment through the membrane during total recirculation of the permeate with a constant permeate flux of 39 $Lm^{-2}h^{-1}$. The %T is the ratio of C_p for each time point over the average retentate concentration in the first 40 minutes. The error bars represent the propagated error of the standard deviation of the permeate concentration as a result of two to four measurements of different dilutions each and of the standard deviation of four retentate concentrations. An exponential decay fit is shown for both repeats (correlation coefficients r^2 = 0.05 for (——, □) and r^2 = 0.001 for (-----, ●). The experiments were conducted under constant retentate flow rate. TMP increased from 0.12 to 0.22 bar throughout the experiments. The two sets of data correspond to two repeats of the same experiment. The volume of lysate was 0.5 L in both experiments.

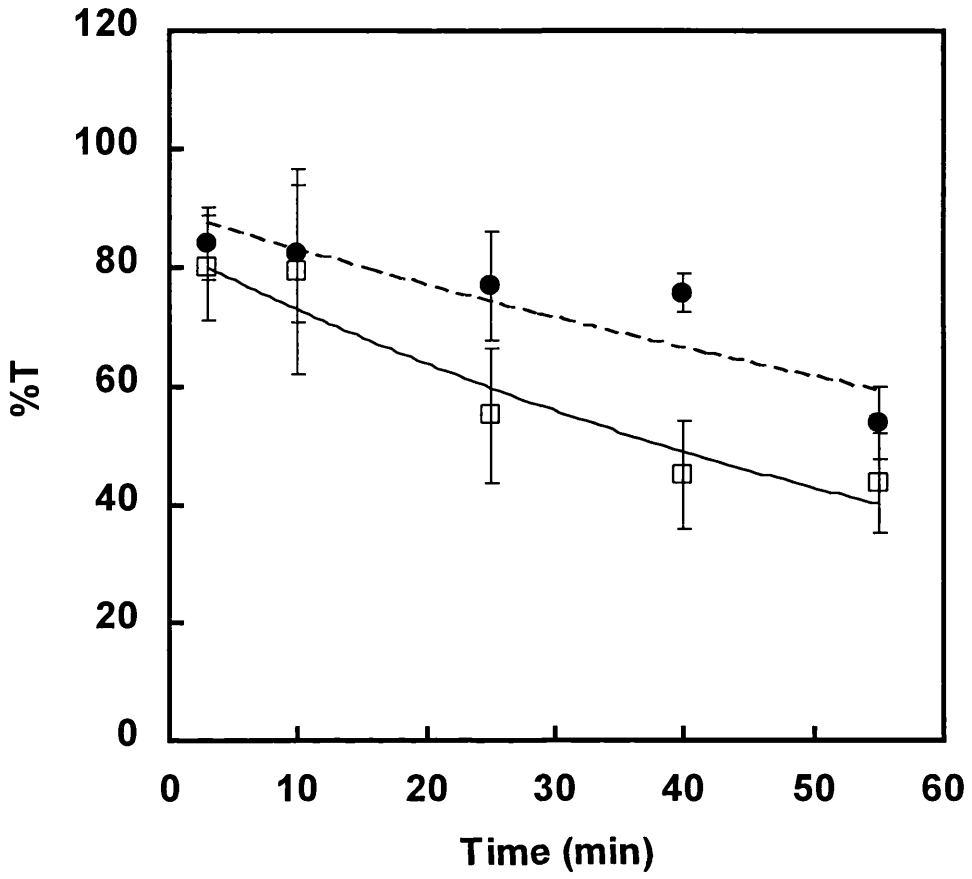


Figure 6.6 Effect of time on the transmission of antibody fragment through the membrane during total recirculation of the permeate with a constant permeate flux of 19 $Lm^{-2}h^{-1}$. The %T is the ratio of C_p for each time point over the average retentate concentration in the first 40 minutes. The error bars represent the propagated error of the standard deviation of the permeate concentration as a result of two to four measurements of different dilutions each and of the standard deviation of four retentate concentrations. An exponential decay fit is shown for both repeats (correlation coefficients r^2 = 0.92 for (——, □) and r^2 = 0.77 for (-----, ●). The experiments were conducted under constant retentate flow rate. TMP oscillated between 0.07 and 0.12 bar throughout the experiments. The two sets of data correspond to two repeats of the same experiment. The volume of lysate was 0.5 L for both experiments.

6.2.3 Constant volume diafiltration

Constant volume diafiltration has often been used to remove soluble proteins from cell lysates (Bailey and Meagher, 2000, Meagher et al. 1994). The advantage of this technique is that it avoids concentration of the non-permeated species, therefore maintaining the physical properties of the mixture being processed.

In the present work lysate clarification was studied using diafiltration against a 150 mM NaCl buffer operating at a controlled flux of 34-38 $Lm^{-2}h^{-1}$ just outside the critical region. The flux presents some variability possibly due to the age of the tubing used in the permeate pump, but it remains effectively constant throughout each experiment.

The monitoring of the clarification step with time shows that % transmission of the Fab' fragment through the membrane decreases from an initial value of 65-70% down to 10% within one hour (Figure 6.7). A first order decay rather than a linear function was assumed for %T as a function of time since the former cannot become negative on extrapolation. As % transmission is the key factor determining the productivity of the separation process, and hence the required filtration area, it is necessary to avoid such low values. Since the same was not observed during total recirculation (Figure 6.5), the decay in %T may not be attributed to fouling.

Other authors have observed a similar disparity between behaviours under total permeate recycle and diafiltration. Bailey and Meagher (2000) attribute the difference to a reduction in the driving force associated with the removal of material from the retentate or changes in the cake layer surface.

Meagher et al. (1994) and Forman et al. (1990) also observed decreasing transmission over time in constant volume diafiltration, although the decrease was slower than in the present study. Also the decrease was accompanied by an increase in TMP, suggesting that compaction of the gel layer was occurring. In the present work TMP increased moderately with time, but the same occurred in the permeate recycle experiment with the same flux, where it was not accompanied by a decrease in %T. This may signify that even if compression of the cake is occurring, it is not responsible for the decrease in %T.

Finally one further group observed a %T decay during diafiltration (Meacle et al., 1999) and attributed it to fouling, since the performance could be improved with backpulsing. Also the levels of %T observed by these authors were similar in total recycle experiments. This was not so in the present case.

Le and Atkinson (1985) also witnessed a decrease in %T during lysate clarification (no diafiltration). According to these authors, this is due to the formation of a secondary membrane by the cell debris and proteinaceous materials. Another aspect affecting %T may be a non-specific adsorption of proteins to cell debris, estimated to affect 20% of the proteins and as a largely reversible process. The authors also mention that the problem should be overcome with washing of the suspension, which is exactly the essence of diafiltration.

Due to the disparity in behaviour between the two operating modes it can be concluded that the decay in transmission of antibody fragment during tangential flow filtration of *E. coli* lysate cannot be fully ascribed to fouling. Transmission may therefore be affected by the concentration of antibody fragment present in the tank (driving force effect) or the concentration of some other species that would also be changing throughout the process. In order to test this hypothesis %T can also be plotted as a function of the number of diafiltration volumes, to expose any differences brought about by the use of different process volumes (Figure 6.8). This representation leads to a slightly better agreement between the two repeats, as can be concluded from the higher correlation coefficient (r^2 = 0.79 instead of 0.63), pointing further to a concentration effect.

Another interesting aspect about the results obtained is the relationship between permeate concentration, C_p, and retentate concentration, C_r (Figure 6.9). The linear relationship seems appropriate, but the line does not cross the origin as would be expected. The straight line can be translated to the left by subtracting a fixed amount of 12 mgL^{-1} to each C_r (15-20% of the initial concentration), so that it will then cross the origin. This would correspond to a situation where %T is constant (given by the gradient of the line) and where the real concentration of antibody fragment available for transmission is in fact less than the measurable value. This could happen in a situation where for example a fixed amount of the antibody fragment is aggregated or

adsorbed to the cell debris and cannot therefore pass through the membrane, but would still be measurable through the analytical methods. Protein aggregates have been reported by other authors to hinder membrane separations (Marshall et al., 1997; Kelly et al., 1993). The impact of this effect will be investigated further in Chapters 7 and 8.

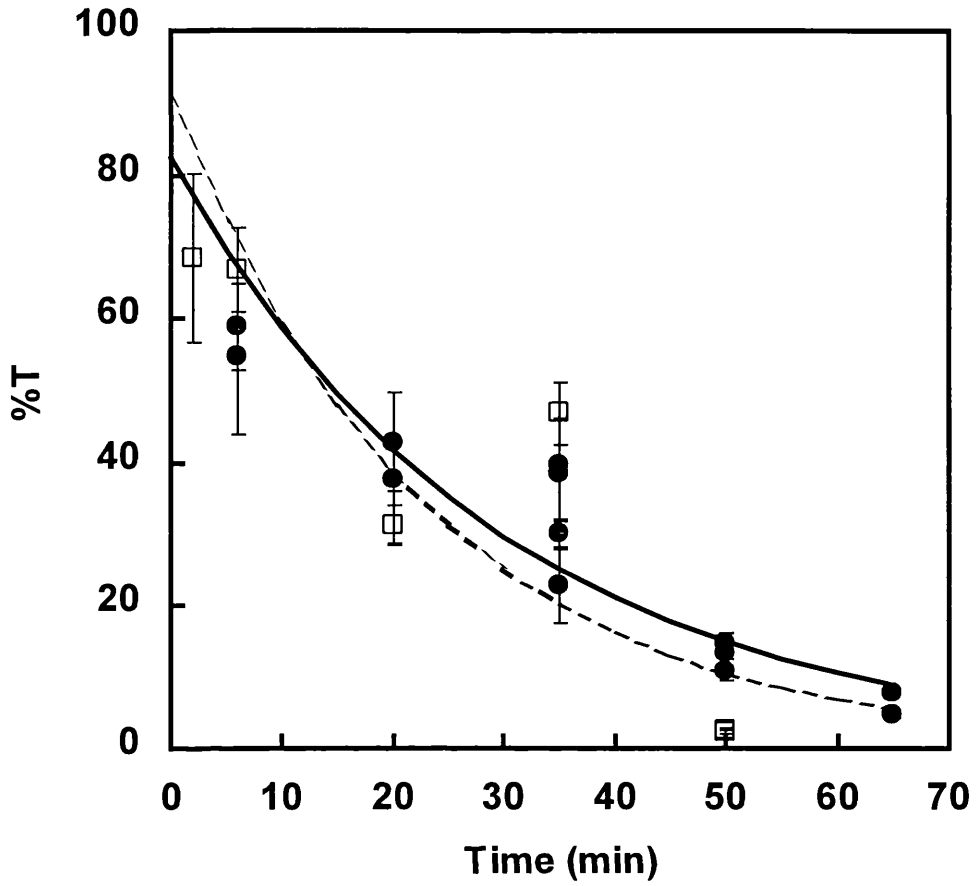


Figure 6.7 Effect of diafiltration processing time on percentage transmission of antibody fragment i.e. ratio of antibody fragment in permeate and retentate. The symbols ● and □ represent two repeats of the experiment. Repeat measurements of the same sample are shown with separate markers. Error bars represent the propagated error from the standard deviation as a result of two to four dilutions of each permeate and retentate sample. An exponential decay fit is assumed (correlation coefficient r^2 = 0.88 (——) or 0.63 (-----) according to whether only the set of results represented by ● or both experiments are used and $\%T=82\ exp^{-0.034t}$ or $\%T = 91\ exp^{-0.043t}$ respectively). Experiments conducted under constant cell concentration (47 g dcw L^{-1}) and constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). TMP increased from 0.07 to 0.10 bar in the experiment represented by ● and varied between 0.07 and 0.16 bar in the experiment represented by □. The flux was kept constant at 38 $Lm^{-2}h^{-1}$ in both experiments. The volume of lysate was 1 L for ● and 0.5 L for □.

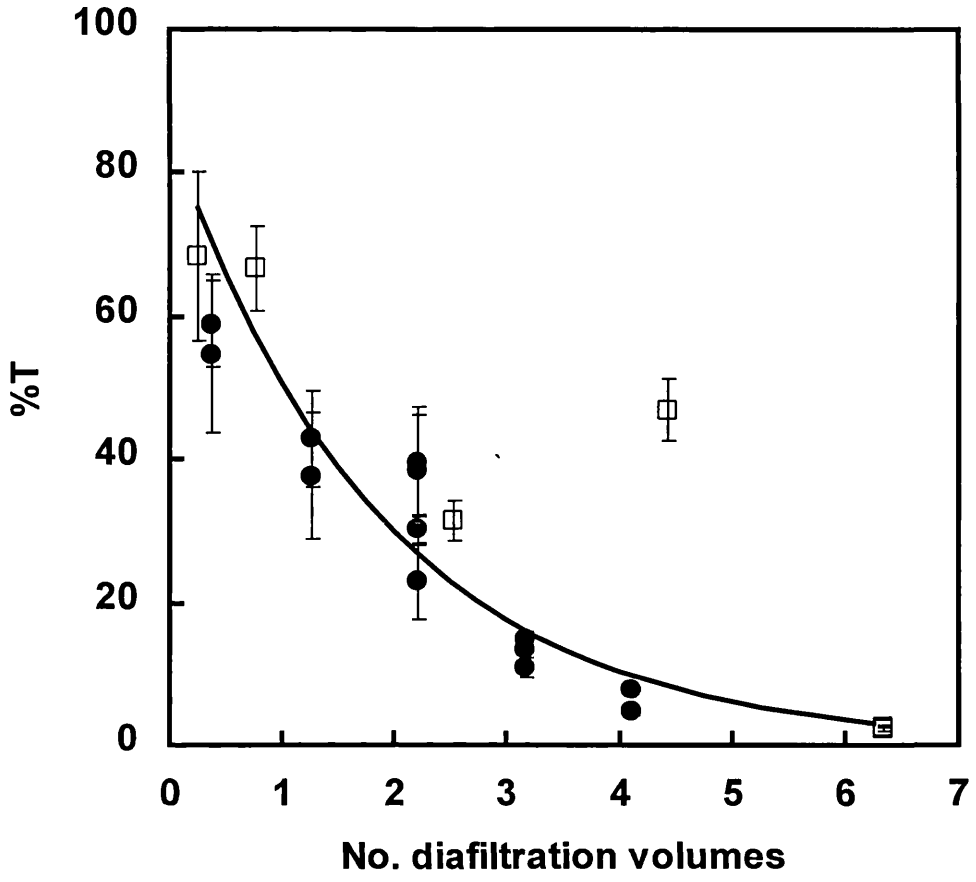


Figure 6.8 Effect of diafiltration volume on percentage transmission of antibody fragment i.e. ratio of antibody fragment in permeate and retentate. The symbols ● and □ represent two repeats of the experiment. Repeat measurements of the same sample are shown with separate markers. Error bars represent the propagated error from the standard deviation as a result of two to four dilutions of each permeate and retentate sample. An exponential decay fit is assumed (correlation coefficient r^2 = 0.79 (——) obtained from both experiments and $\%T=86\ exp(-0.526N_D)$, where N_D is the number of diafiltration volumes). Experiments conducted under constant cell concentration (47 g dcw L^{-1}) and constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). TMP increased from 0.07 to 0.10 bar in the experiment represented by ● and varied between 0.07 and 0.16 bar in the experiment represented by □. The flux was kept constant at 38 $Lm^{-2}h^{-1}$ in both experiments. The volume of lysate was 1 L for ● and 0.5 L for □.

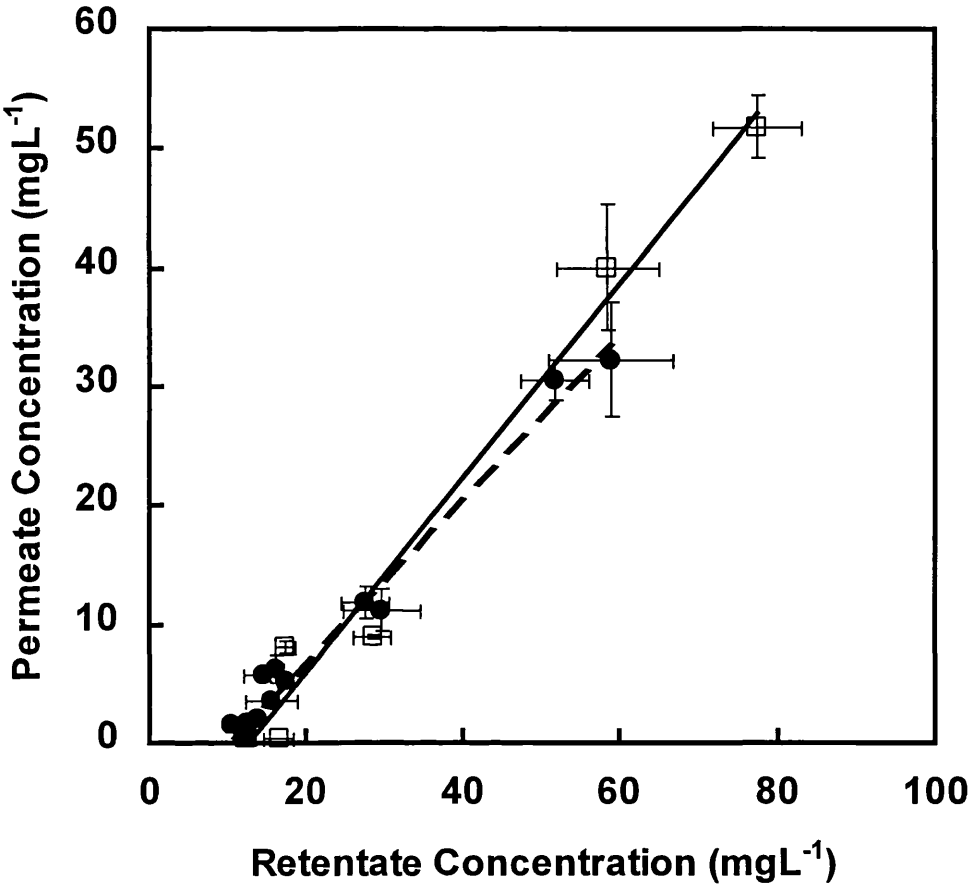


Figure 6.9 Permeate concentration (C_p) as a function of retentate concentration (C_r) during diafiltration. The symbols ● and □ correspond to the same repeats as in Figure 6.7. Repeat measurements of the same sample are shown with separate markers. Error bars represent the standard deviation as a result of two to four dilutions of each permeate and retentate sample. A least squares linear decay fit is assumed for each repeat of the experiment: Cp = 0.67 Cr – 6.5, r^2 = 0.98 for (- - - -, ●) and Cp = 0.81 Cr – 10.4, r^2 = 0.98 for (——, □). Experiments conducted under constant cell concentration (47 g dcw L^{-1}) and constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). TMP increased from 0.07 to 0.10 bar in the experiment represented by ● and varied between 0.07 and 0.16 bar in the experiment represented by □. The flux was kept constant at 38 $Lm^{-2}h^{-1}$ in both experiments. The volume of lysate was 1 L for ● and 0.5 L for □.

In order to establish the reason for the decay in transmission, experiments were performed with different diafiltration buffers, to allow the evaluation of effects such as ionic strength or pH.

The first diafiltration was performed with lysis buffer. This was to simulate the conditions during total permeate recycle. Figure 6.10 shows that %T decays as a function of time as was observed in Figure 6.7.

The same observation can be made for diafiltration with NaCl 100 mM, which has a value of conductivity more similar to that of the lysate than the diafiltration buffer used in the experiments in Figure 6.7, NaCl 150 mM (see Table 6.1). In fact a faster decay of %T may even be occurring in this case, as can be inferred from the more negative exponential decay factor (a = -0.1 min^{-1} for NaCl 100 mM instead of a = -0.04 min^{-1} for NaCl 150 mM). However this value of a may be misleading since the value of %T at t=0 given by the same fit is much higher than 100, which does not make physical sense, despite the high correlation factor.

Table 6.1 Conductivity measurements for the lysate and different buffers.

	Conductivity ($m\Omega cm^{-1}$)
Lysate	10.9
NaCl 150 mM	16.8
NaCl 100 mM	11.6
Lysis Buffer	9.0

On the other hand, according to Le and Atkinson (1985), %T should increase with increasing buffer ionic strength. One interpretation for that fact is an increase of the effective size of the protein due to swelling or association with other proteins at lower ionic strength. Although this would seem inconsistent with the readings of conductivity of the lysate and the total recycle experiment, it may be worth investigating the impact on transmission of a higher NaCl concentration.

Additionally a pH effect was also ruled out since the pH of both the lysis buffer and the diafiltration buffer are close to neutrality, once again not presenting a fundamental

difference between the two modes of operation. However Le and Atkinson (1985) do report that %T is highest at the pI of the protein. It may therefore be worth checking the impact on %T of performing diafiltration with a buffer at pH 8.3, which is the isoelectric point of the antibody fragment (Bowering, 2001).

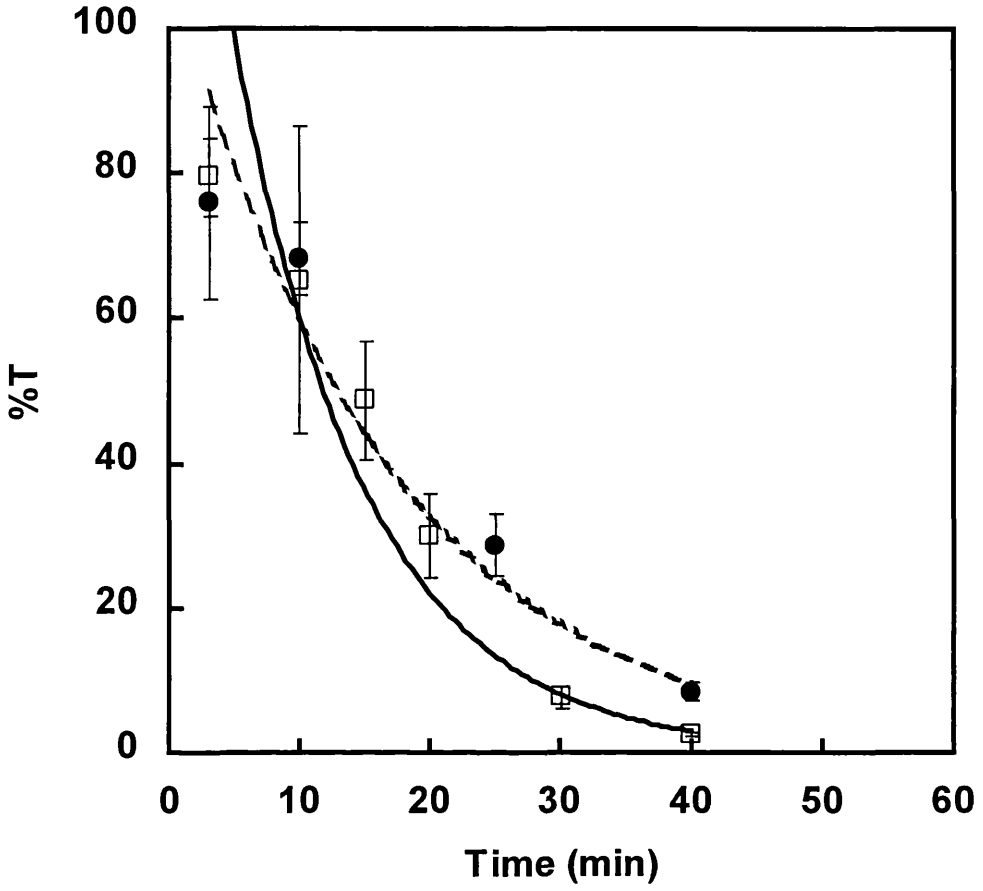


Figure 6.10 Effect of the use of a different diafiltration buffer on the percentage transmission of antibody fragment. The buffers used were lysis buffer (●) and NaCl 100 mM (□). Error bars are the propagated error from the standard deviation as a result of two to three dilutions of each permeate and retentate sample. An exponential decay fit is assumed for both experiments ($\%T=109\ exp^{-0.061t}$ and correlation coefficient $r^2 = 0.97$ (- - - -) for the experiment with lysis buffer and $\%T = 163\ exp^{-0.1t}$ and correlation coefficient $r^2 = 0.96$ (——) for the experiment with NaCl 100 mM. Experiments conducted under constant cell concentration (47 g dcw L^{-1}) and constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). TMP varied between 0.14 and 0.21 bar in the lysis buffer experiment and between 0.17 and 0.22 bar in the NaCl 100 mM experiment. The flux was maintained at 38 $Lm^{-2}h^{-1}$ throughout both experiments. The volume of lysate was 0.5 L for both experiments.

Diafiltration was also performed with the permeate pump set at a lower value of flux (25 LMH instead of 39 LMH), as shown in Figure 6.11. This experiment confirms that the decay in %T was not due to being close to the critical flux, since no significant difference was observed.

Finally diafiltration was repeated with a higher volume of lysate, V = 1.3 L (Figure 6.12). With these conditions it took 60 minutes to reach 2.5 diafiltration volumes, against 20 minutes taken by the process performed with 0.5 L of lysate (□ in Figure 6.7). Despite this time difference, the level of %T observed at this value of diafiltration volume is very similar for both cases, at approximately 20% (Figure 6.12 and □ in Figure 6.8). This indicates that time alone is not responsible for the decrease in %T, at least during the first hour, as was already expected from the experiments made with total permeate recycle. It is therefore likely that %T is mainly affected by the concentration of some species present in the lysate and to which the membrane is at least semi-permeable. Alternatively the hypothesis that some product is not available for transmission is also a suitable explanation (as concluded from Figure 6.9).

The lower value of %T for t = 2 minutes observed in Figure 6.12 is possibly due to dilution of the permeate from the conditioning buffer, present in the system before addition of the lysate.

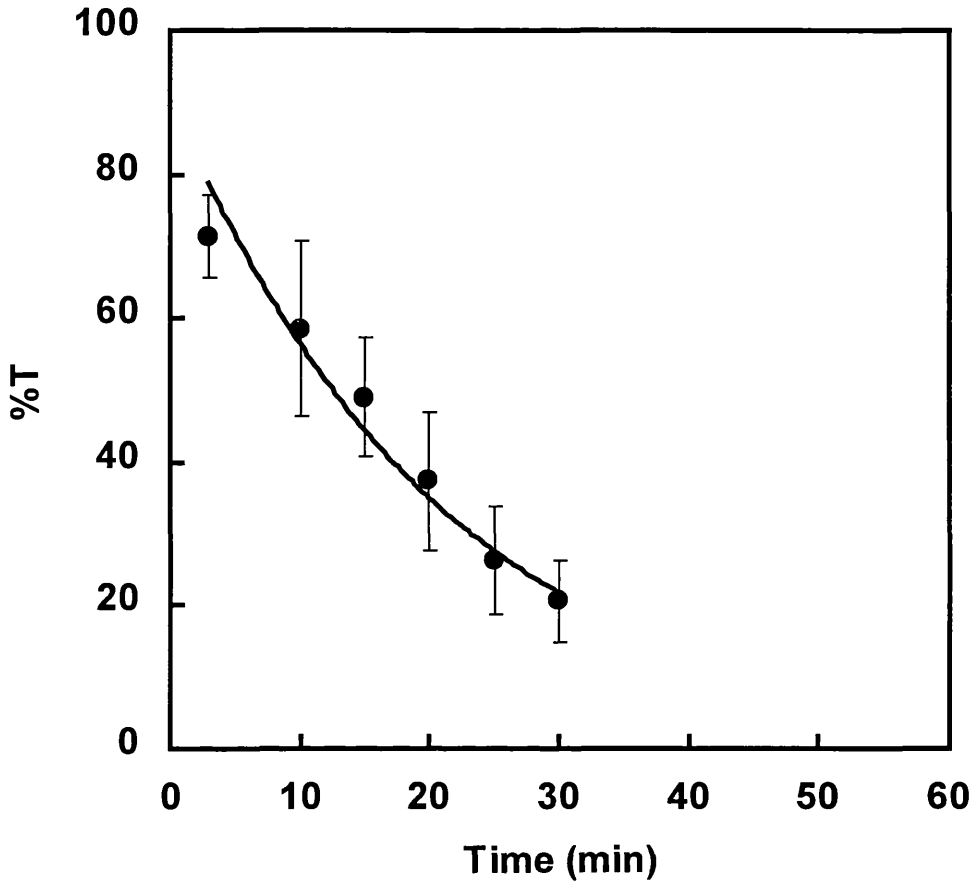


Figure 6.11 Effect of the use of a lower diafiltration flux (25 $Lm^{-2}h^{-1}$) on the percentage transmission of antibody fragment (as a function of time). Error bars are the propagated error from the standard deviation as a result of three to four dilutions of each permeate and retentate sample. An exponential decay curve was assumed ($\%T = 91e^{-0.048t}$, $r^2 = 0.97$). Experiment conducted under constant cell concentration (47 g dcw L^{-1}) and constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). TMP remained at 0.16-0.17 bar and the flux was kept constant at 25 $Lm^{-2}h^{-1}$ throughout the experiment.

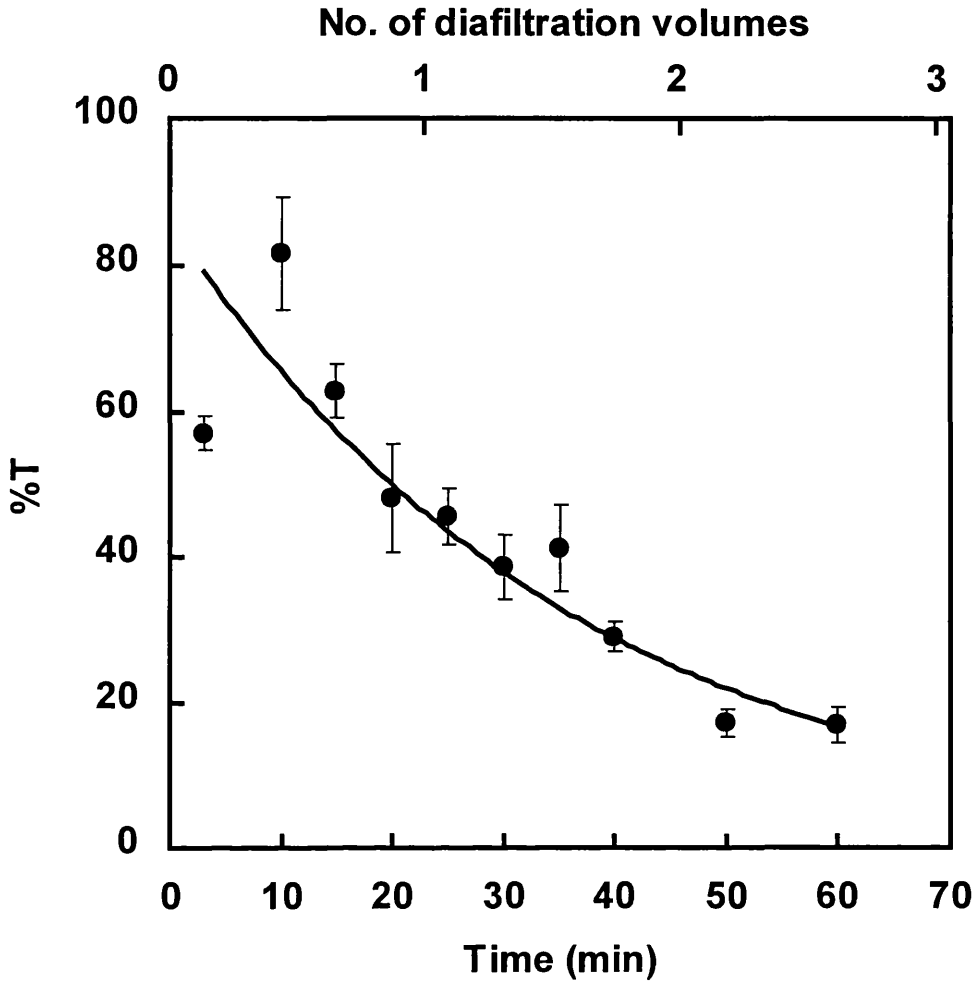


Figure 6.12 Effect of the use of a higher lysate volume (V = 1.3 L) on the percentage transmission of antibody fragment (as a function of time and of the number of diafiltration volumes). Error bars are the propagated error from the standard deviation as a result of three to four dilutions of each permeate and retentate sample. An exponential decay curve was assumed ($\%T = 86e^{-0.027t}$, $r^2 = 0.88$). Experiment conducted under constant cell concentration (47 g dcw L^{-1}) and constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). TMP increased from 0.19 to 0.22 bar and the flux was kept constant at 34 $Lm^{-2}h^{-1}$ throughout the experiment.

6.2.4 Diafiltration of spun-down lysate

An experiment was run to identify the influence of the presence of cell debris on the diafiltration performance (%T). Lysate was spun down to remove the spheroplasts and cell debris and the supernatant was used as the feed in constant volume diafiltration. Contrary to experiments with whole lysate, the level of %T was still above 50% after 1 hour of operation (Figure 6.13). This result rules out the possibility that %T might be going down solely as a result of a decrease in the concentration driving force, as pointed out in section 6.2.3.

A further aspect that is interesting to note is that in this case there would be no detrimental effect of a different ionic strength or pH. If indeed the protein molecules increase in volume at a lower ionic strength, this only appears to affect the %T when in the presence of cell debris. One possible explanation for that might be that with the whole lysate a secondary layer composed of cell debris and proteins forms on the surface of the membrane (Le and Atkinson, 1985), resulting in a reduced effective molecular weight cut-off.

Van Reis et al. (1997) claim that protein aggregation is concentration dependent, since it is a result of protein-protein interactions. If one assumes that it can also be induced by the presence of cell debris, this might explain the non-availability of some protein during the diafiltration of lysate. This "non-available" protein would become increasingly important as the "available" protein is removed, as opposed to diafiltration of lysate supernatant, where aggregation would not occur due to the absence of debris. Also in the case of total permeate recycle the overall protein concentration is constant and so the proportion of "non-available" protein would remain unchanged and small throughout the process. This could explain why %T is less than 100% in the total recycle experiment.

Figure 6.14 does show that when spun down lysate is used, the totality of the antibody fragment present in the retentate side is "available" for transmission, since the plot of C_p vs. Cr crosses the origin. The decrease in %T as a result of fouling can be seen from an enlargement of this figure in the lower concentration range (Figure 6.15). The experimental points arch slightly, showing the gradient is lower nearer the origin.

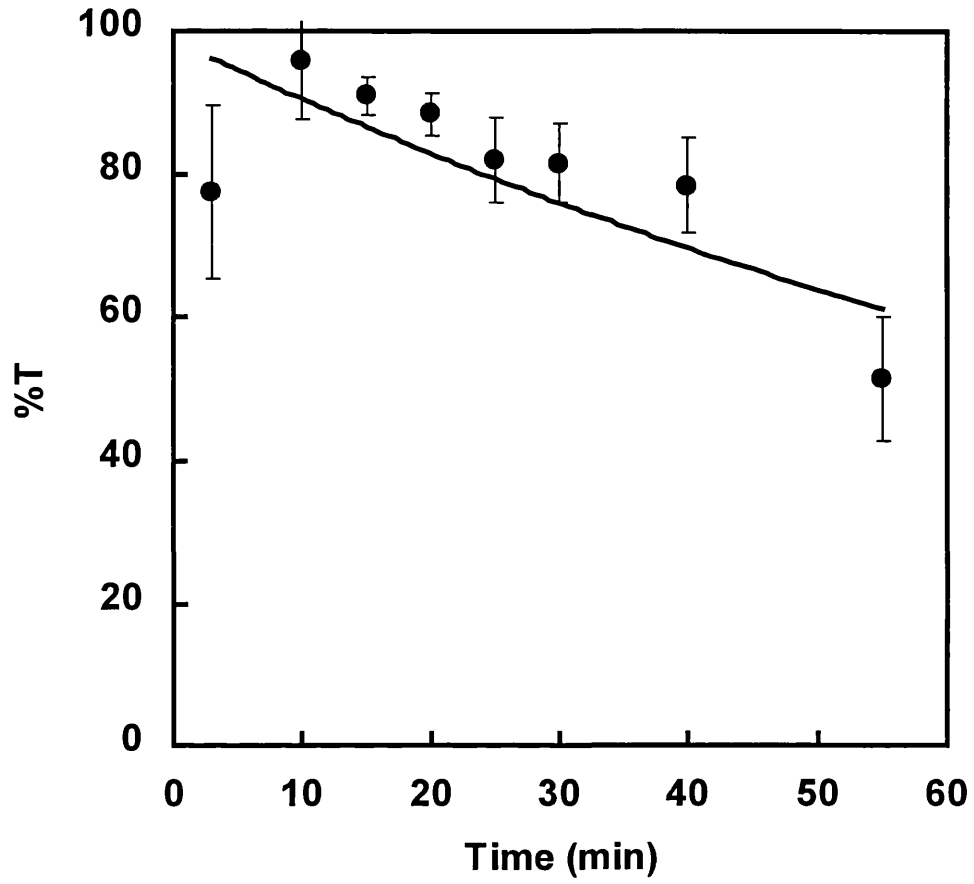


Figure 6.13 Effect of diafiltration processing time on percentage transmission of antibody fragment, experiment done with spun down lysate. Error bars are the propagated error from the standard deviation as a result of two to four dilutions of each permeate and retentate sample. An exponential decay curve was assumed (%T = $99e^{-0.009t}$, r^2 = 0.59). Experiments conducted under constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). TMP varied between 0.07 and 0.14 bar during the experiments and the flux was kept constant at 35 $Lm^{-2}h^{-1}$. The volume of spun down lysate (feed) was 0.5 L.

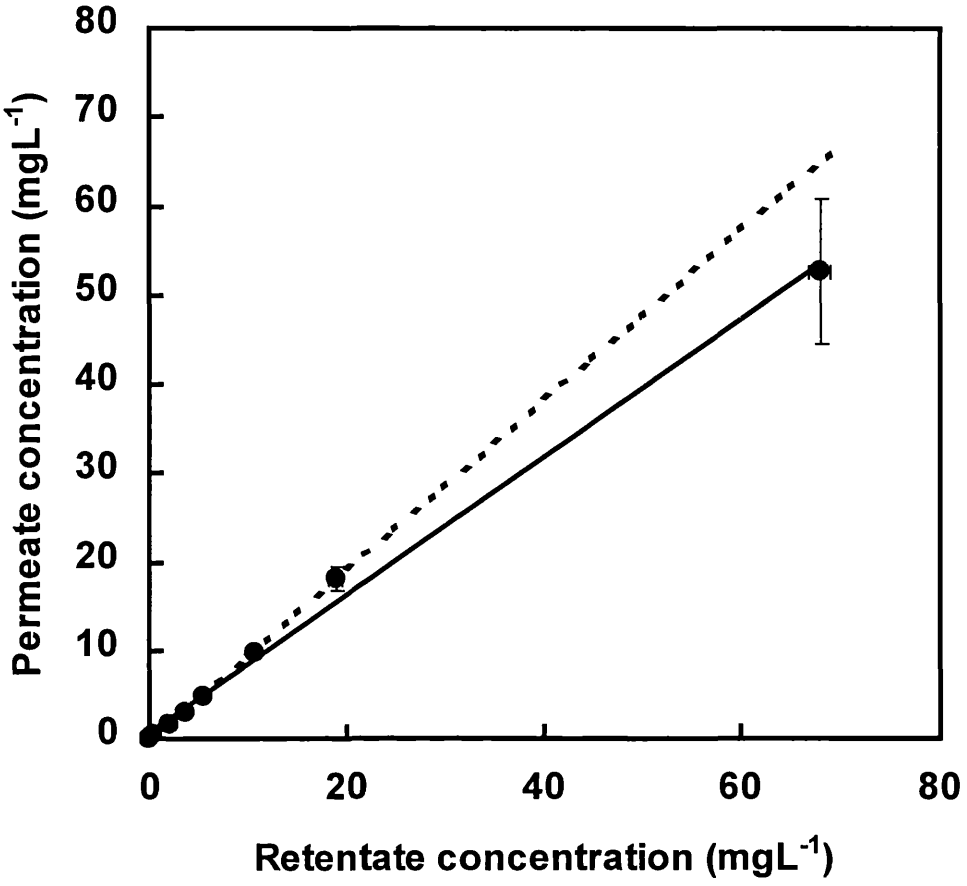


Figure 6.14 Permeate concentration (C_p) as a function of retentate concentration (C_r) during diafiltration of spun-down lysate. Error bars represent the standard deviation as a result of two to four dilutions of each permeate and retentate sample. A least squares linear decay fit was assumed: Cp = 0.78 Cr + 0.7, r^2 = 0.995 calculated with all the points (—) or Cp = 0.96 Cr – 0.3, r^2 = 0.999 calculated excluding the point for higher concentration (corresponding to t=3 minutes in Figure 6.13) as the value of C_p may have been underestimated due to initial dilution (- - - -). Experiments conducted under constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). TMP varied between 0.07 and 0.14 bar during the experiments and the flux was kept constant at 35 $Lm^{-2}h^{-1}$. The volume of spun down lysate (feed) was 0.5 L.

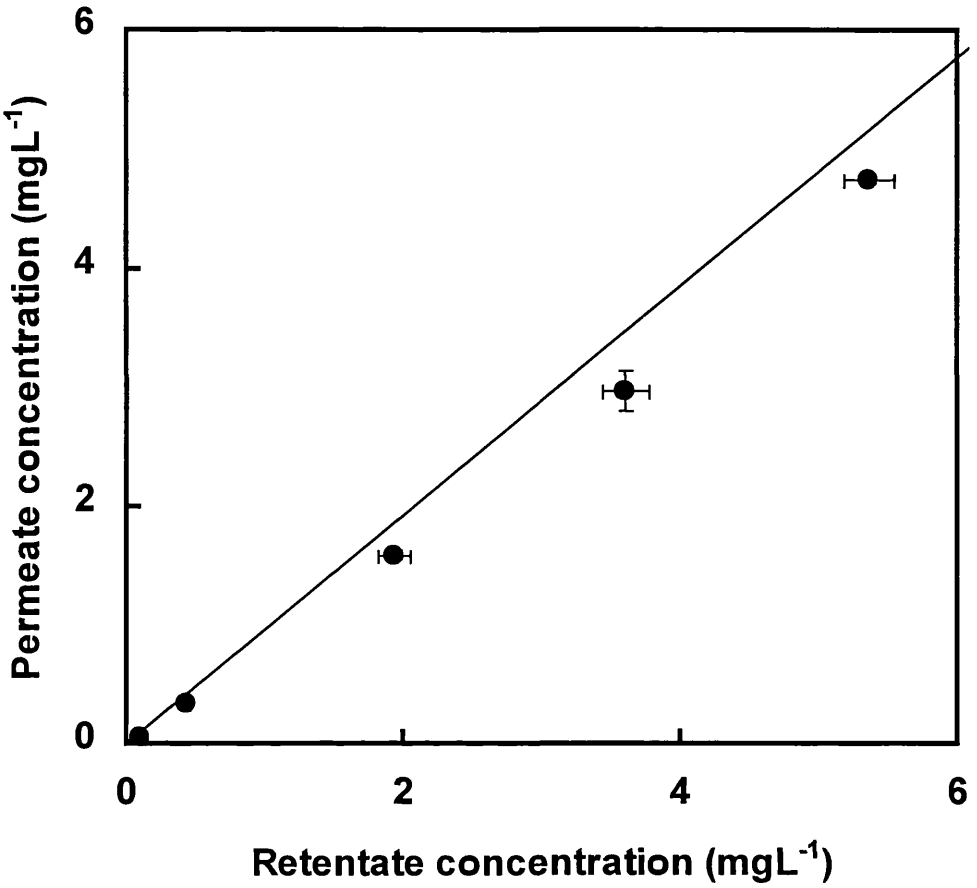


Figure 6.15 Permeate concentration (C_p) as a function of retentate concentration (C_r) during diafiltration of spun-down lysate (enlargement of Figure 6.14 for the lower range of concentrations). Error bars represent the standard deviation as a result of two to four dilutions of each permeate and retentate sample. The straight line represents the least squares linear decay fit represented in Figure 6.14 by (- - - -) but adjusted to cross the origin: Cp = 0.96 Cr.

6.3 Conclusions

Several conclusions can be made from this set of experiments. First of all the percentage transmission presents a very fast decay during diafiltration of *E. coli* lysate. To compensate for the low performance, the membrane area required for the separation needs to be large, so that the process can be conducted within a reasonable period of time. This is detrimental from an economic point of view, particularly in a disposable process, where the membrane has to be replaced at each new batch.

The main contribution to the decrease in transmission cannot be exclusively attributed to fouling since the decrease is not observed as strongly with total permeate recycle. In fact the transmission appears to drop as a result of a decrease in the concentration of the species being removed from the system, possibly that of Fab'. A possible explanation is that there may be an amount of product that is not available for transmission. This "non-available" material could be in the form of aggregates assayable with ELISA, but not present in the spun down material, since a %T decay is not observed in this case. Another possibility would be adsorption of Fab' to the cell debris.

The hypothesis that the reduction in %T is due to a decrease in the concentration driving force does appear unlikely since it does not occur with spun down lysate. It could also be that a proportion of Fab' swells due to a change in the environment, but this would have to be an irreversible effect, since the quantity of "non-available" Fab' is apparently constant with time, as can be hinted from Figure 6.9. To confirm this fully, experiments can be done where the diafiltration buffer has a higher ionic strength or a pH close to 8.3, which is the isoelectric point of the Fab' antibody fragment. If swelling does occur then the effect should be apparent in the spun down lysate experiments, unless there is less hindrance to the passage of the swollen proteins (no secondary membrane formed by the cell debris).

Some fouling occurs during diafiltration of spun-down lysate, and in this case it should be the sole mechanism responsible for the decrease in %T. Intuitively one might expect the fouling to be more severe in the presence of cell debris, but these may also form a cake that prevents the formation of a protein layer, thereby improving the overall transmission, as Kuberkar and Davis (1999) observed when yeast is added to a

BSA solution. This also highlights the need for more membrane filtration studies with real process streams instead of idealised protein solutions.

One further reason for the results observed could be that material might get released from the cell debris during centrifugation of the samples, but this is unlikely due to negligible level of shear damage induced by a micro-centrifuge (Boychyn, 2000). The freeze/thawing of the samples prior to analysis could potentially also lead to the release of more Fab', that would still have been intracellular at the time of the MF experiment. This is however unlikely to have a strong impact, since three hours of periplasmic release at 60°C allow the release of more than 95% of the Fab' available (Bowering, 2000).

The observation that some material may not be "available" for filtration is important and may go some way to explaining other results reported in the literature. The fact that even small amounts of "non-available" material can lead to such significant drops in apparent transmission means that any membrane optimisation procedure needs to be approached with care. This result is also crucial in determining how best to use the membranes in a disposable fashion as a true understanding of transmission is essential in knowing when to cease diafiltration on economic grounds.

Where transmission decay is unavoidable and due to fouling new approaches will need to be developed to minimise its effects. A strategy for membrane area optimisation will be described in Chapter 7, followed by experimental evaluation in Chapter 8.

Chapter 7 Modelling of transmission and of a membrane regeneration strategy

7.1 Introduction

The fast decrease of transmission with time observed in Chapter 6 is clearly detrimental in terms of required membrane areas. Although some level of decrease, as a result of fouling, can never be totally avoided in the filtration of biological materials, it is possible to minimise the extent to which this happens.

The first objective of this chapter was to find models that describe the observed transmission as a function of time (section 7.2). Subsequently these models were used to predict the effect of regeneration steps on the overall performance and hence to estimate membrane area savings (section 7.3). The regeneration steps consist here of interrupting the process at regular intervals and performing membrane rinsing for a short period of time with diafiltration buffer. This strategy was chosen as it does not introduce any foreign compounds to the system, as would be the case with chemical cleaning, and allows for an immediate resumption of the process once it has been performed.

The theoretical impact on the running costs and NPV of the disposable option will also be calculated with the models developed (section 7.4). This will show the economic importance of such a strategy in a disposables-based process.

7.2 Transmission models

7.2.1 Significance

In order to improve the transmission of product through the membrane it is helpful to be able to represent transmission behaviour as a function of time with the aid of a model. For that purpose it will be important to understand which factors affect transmission. Usually the decrease of transmission is due to fouling effects on the surface of the membrane and is solely dependent on the processing time (for given process conditions), as is assumed in Model 1 (section 7.2.2). Results reported in

Chapter 6 gave some indication that the decrease in transmission may not be exclusively due to processing time. For that reason a model that simply expresses %T as a function of time may not be complete. Model 2, presented in section 7.2.3, therefore attempts to include the product concentration as a further variable, through the introduction in the model of the volume of feed to be processed and the flux used.

7.2.2 Model 1: %T as a function of time

In the first model it is assumed that the decay of %T observed in Figure 6.7 (Chapter 6) is exclusively due to fouling.

Assumptions:

- The tank is well mixed.
- V is constant, inflow of diafiltration buffer equal to flux.
- $C_f \approx C_r$.
- There is no loss of product (Fab') throughout the process.

Membrane area is the key variable and it can be obtained from a mass balance for the system (Figure 7.1):

$$0 - JAC_P = V\frac{dC_F}{dt}$$

Equation 7.1

Additionally the observed transmission of the product through the membrane is expressed according to:

$$T_{obs} = \frac{C_p}{C_B}$$

Equation 7.2

C_B is the concentration in the bulk of the liquid outside the membrane (see Figure 1.2, Chapter 1), and can be approximated by the concentration in the retentate, C_R.

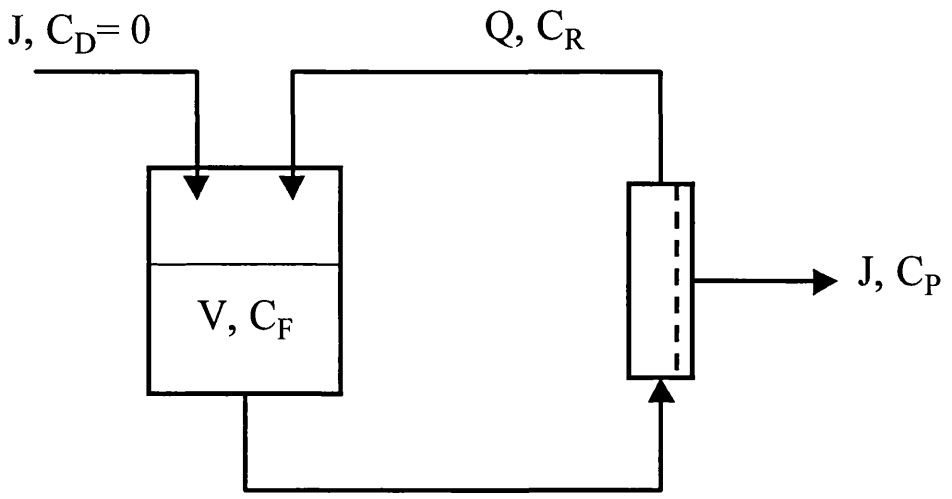


Figure 7.1 Constant volume diafiltration - system variables.

Model 1 assumes that transmission follows a first order decay:

$$T_{obs} = T_0 e^{at}$$

Equation 7.3

Equation 7.1 can be integrated:

$$-JA\int_0^t T_{obs}\,dt = V\int_{C_{R0}}^{C_R}\frac{dC_R}{C_R} = V\ln\left(\frac{C_R}{C_{R0}}\right) = V\ln(1-Y)$$

Equation 7.4

where Y is the yield of the process.

The membrane area can therefore be expressed as a function of the different variables of the system:

$$A = \frac{-V\ln(1-Y)}{J\frac{T_0}{a}(e^{at}-1)}$$

Equation 7.5

The values of T_0 and a can be obtained from the experimental data. For example in a lysate diafiltration experiment (Figure 6.7), $T_0 = 0.91$ and $a = -0.04$ min^{-1}. These values will be used below in the membrane area optimisation studies (section 7.3.2).

7.2.3 Model 2: %T as a function of time and hindered product effect

In this second model it is assumed that not all the product (Fab' antibody fragment) is available for transmission, but that it is available for measurement (see Chapter 6). This means that the observed transmission does not correspond to the actual transmission that is occurring in the system. Possible reasons for this have been outlined in Chapter 6 and include: that some Fab' antibody fragment may be adsorbed onto the cell debris surface but released by the analytical procedure; or that it is aggregated and cannot pass through the membrane but is still assayable through ELISA, etc.

The assumptions made in section 7.2.2 are still valid here, but some further assumptions have to be added:

- The absolute amount of Fab', C_r, is not available for transmission:

$$C_r = C_r^A + C_r^N$$

Equation 7.6

C_r^A is the fraction of Fab' available for transmission and C_r^N is the fraction of Fab' not available for transmission.

- A further concept of transmission has to be introduced, that is the real transmission happening in the system, higher than the observed transmission:

$$T_{real} = \frac{C_p}{C_r^A}$$

Equation 7.7

- The membrane fouls slowly:

$$T_{real} = T_0' e^{a't}$$

Equation 7.8

- C_r^N is considered to be constant throughout the filtration process.

Substituting Equation 7.7 and Equation 7.6 in Equation 7.1:

$$-JAC_r^A T = V\frac{d}{dt}(C_r^A + C_r^N)$$

Equation 7.9

As C_r^N is constant,

$$\frac{d}{dt}(C_r^A + C_r^N) = \frac{dC_r^A}{dt}$$

Equation 7.10

And so, after substitution and integration:

$$-\frac{JA}{V}\frac{T_0'}{k}(\exp(a't) - 1) = \ln(\frac{C_r^A}{C_{r0}^A})$$

Equation 7.11

Transmission (observed, as defined in Equation 7.2) can be rewritten as:

$$T_{obs} = \frac{C_{r0}^A \exp(\frac{-JA}{V}\frac{T_0'}{a'}(\exp(a't) - 1))}{C_r^N + C_{r0}^A \exp(\frac{-JA}{V}\frac{T_0'}{a'}(\exp(a't) - 1))} T_0' \exp(a't)$$

Equation 7.12

Assuming x is the initial fraction of non-available Fab' then:

$$C_{r0}^N = C_r^N = xC_{r0}$$

$$C_{r0}^{A} = (1-x)C_{r0}$$

Equation 7.13

Replacing Equation 7.13 in Equation 7.12 and simplifying:

$$T_{obs} = \frac{(1-x)\exp(\frac{-JA}{V}\frac{T_0'}{a'}(\exp(a't)-1))}{x+(1-x)\exp(\frac{-JA}{V}\frac{T_0'}{a'}(\exp(a't)-1))}T_0'\exp(a't)$$

Equation 7.14

Equation 7.14 above is the definition of Model 2. The difficulty is then to find the correct expression for T_{real}. The variables T_0' and a' can be estimated from experimental data where the effect of non-available Fab' would not be present. For example in total permeate recycle experiments the impact of the "non-available" product is minimized by the fact that its concentration is constant. Diafiltration with spun-down lysate is a second possibility, where the effect of the cell debris is absent.

Figure 7.2 shows the fit of Equation 7.14 to the experimental data presented in Figure 6.12, assuming T_0' = 99% and a' = -0.009, as obtained from the spun-down lysate experiment (Figure 6.13). This was preferred to the total permeate recycle experiment due to the low correlations factors obtained for the latter. The value of x obtained from a least squares fit is 0.19. This means that 19% of the initial amount of Fab' is not available for transmission, and that this quantity remains unchanged throughout the process.

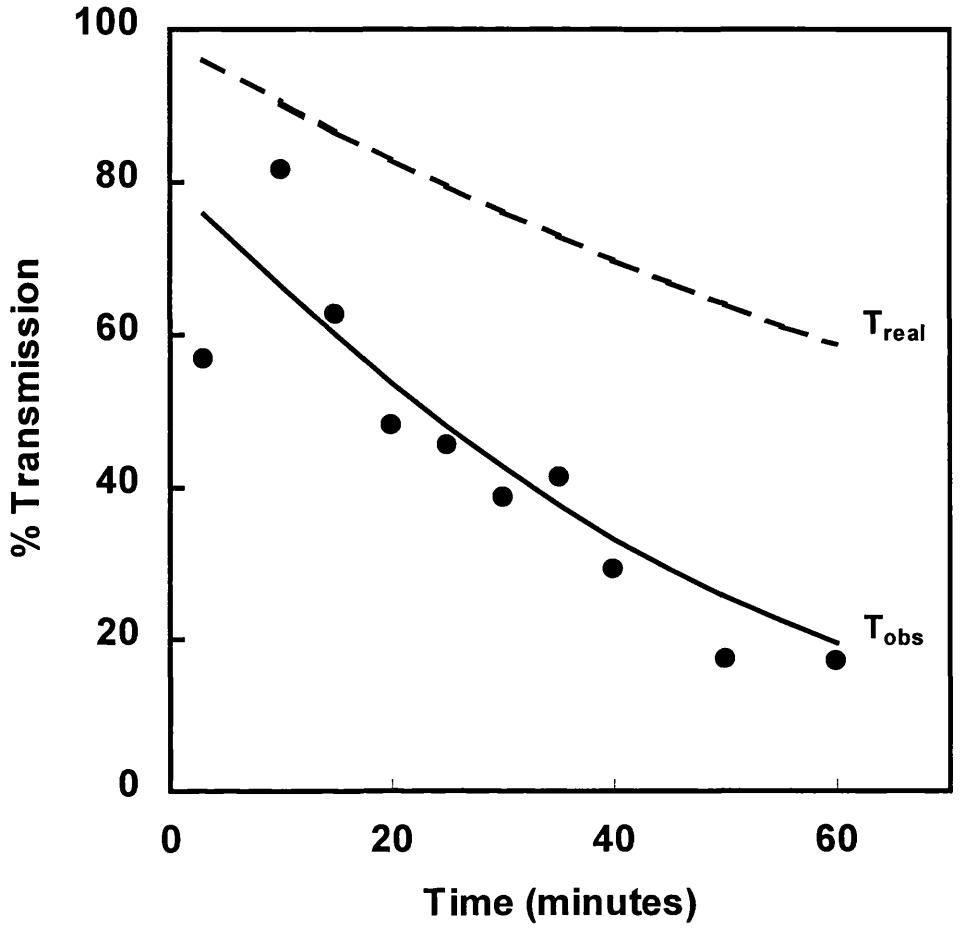


Figure 7.2 Theoretical fit of the experimental values of T_{obs} of Figure 6.12 with Model 2, Equation 7.14 (—). The theoretical curve (r^2 = 0.89) was obtained using the same variables as those used in the experiment itself: V = 1.4 L, A = 0.1 m^2, J = 34 $Lm^{-2}hr^{-1}$ (disregarding the value of %T for t = 3 minutes). T_{real} is also shown (- - -), according to Equation 7.8, with the values of T_0' and a' taken from the spun down lysate experiment.

Table 7.1 shows the value of x for several experiments, where x = 0.2 is the average value. It can be seen that there is some variability in the values obtained, especially for experiment □ of Figure 6.7, although the value does get closer to the other values when the outlying point is excluded from the calculations, as well as improving the value of r^2. One cause for the variability of x might be the variability of the lysate.

Experiment	x	r^2
Figure 6.7 (●)	0.23	0.84
Figure 6.7 (□)	0.13 (0.18)	0.64 (0.98)
Figure 6.11	0.21	0.99
Figure 6.12	0.21 (0.19)	0.80 (0.89)

Table 7.1 Initial fraction of Fab' not available for transmission and r^2 calculated for different sets of experimental results and considering T_{real} is based on the experimental values of T_0' and a' obtained for the spun down lysate diafiltration experiment (Figure 6.13). The values inside brackets for experiment □ were obtained considering the point at t=35 minutes is an outlier and those for Figure 6.12 were obtained disregarding the first point (low value of transmission possibly due to dilution with conditioning buffer).

7.3 Membrane area optimisation

7.3.1 Strategy

The objective of this modelling exercise is to calculate the area necessary to process a set volume of lysate (V) in a set period of time (t). It is assumed that the level of transmission decreases with time following one of the models described in 7.2. Furthermore it is assumed that the levels of transmission can be recovered fully or at least partially by a regeneration step of duration t_R. After regeneration the process is resumed with fresh lysate. This strategy will allow the advantage of higher initial transmission values to be exploited.

7.3.2 Membrane area savings calculated with Model 1

The first approach considered %T follows a first order decay as in Equation 7.3. This corresponds to a scenario where all Fab' is available for recovery and there is a sharp decline in transmission with time. The clarification step will consist of N processing steps of duration t_P interspaced by the regeneration steps, of duration t_R. The times for processing and rinsing, t_P and t_R are constant for one particular process. Hence the total clarification time, t, is given by:

$$t = Nt_p + (N-1)t_R$$

Equation 7.15

The total volume of feed is divided into N identical aliquots of volume V/N, so the area is given by:

$$A = \frac{-V\ln(1-Y)}{NJ\frac{T_0}{a}(e^{at}-1)}, \text{ for } t = t_P.$$

Equation 7.16

The variables to be used in the equation above were chosen so as to simulate a pilot scale process: V = 100 L, yield = 96% in a total process time of 240 minutes. The results are presented graphically in Figure 7.3. It can be seen that for rinsing stages of duration of 10 minutes it is theoretically possible to reduce the required membrane area by up to 80% (8 regeneration stages). Even 3 regeneration stages are enough to allow a 70% saving in membrane area.

The membrane area savings are accompanied by diafiltration buffer savings, as illustrated in Figure 7.4 for 10-minute rinsing steps. The reason for this is that the volume of diafiltration buffer is dictated by the flow of liquid through the membrane (constant volume mode), which in turn is dependent on the membrane area according to:

$$V_D = NJAt_P$$

Equation 7.17

On the other hand the regeneration stage also requires diafiltration buffer, which will increase with the number of regeneration steps. Figure 7.4 also shows the savings in diafiltration buffer achievable after deduction of the volume increase associated with the rinsing, assuming each rinsing step requires a volume of buffer equivalent to the tank volume, i.e. V/N. As this volume decreases with the number of steps, the total volume of buffer needed for rinsing is almost independent of the number of steps.

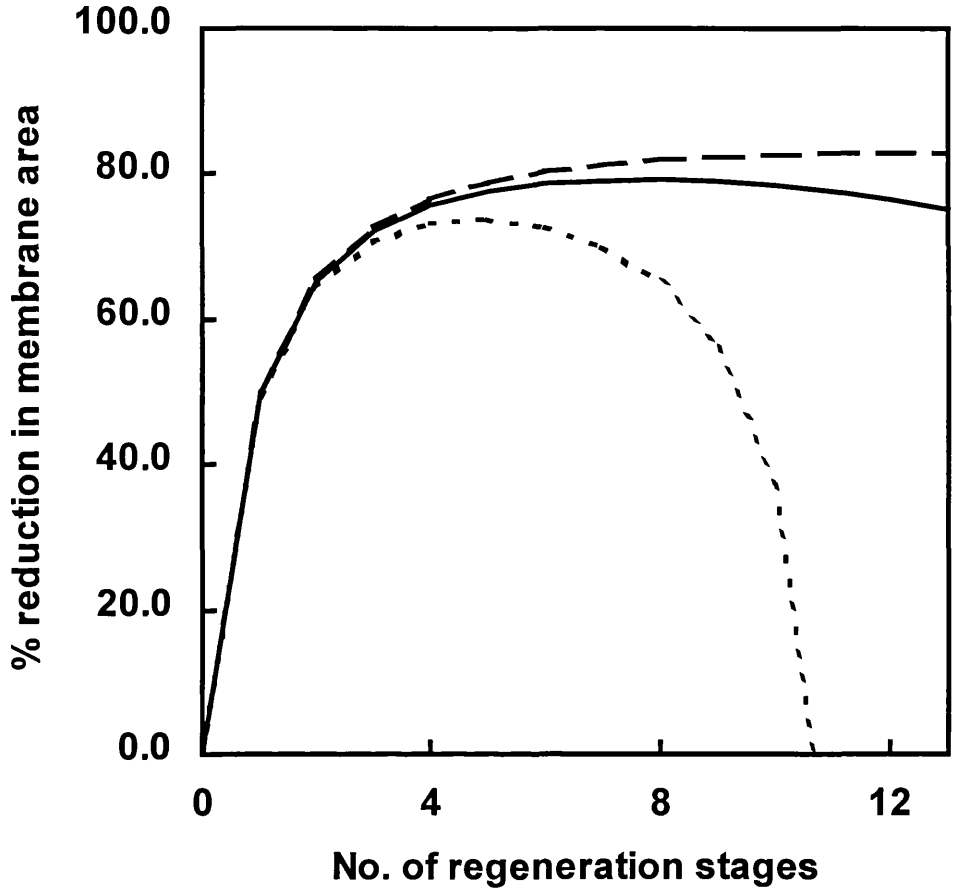


Figure 7.3 Percentage reduction in membrane area as a function of the number of regeneration stages, calculated for different regeneration times: t=5 minutes (— —), t=10 minutes (—),and t=20 minutes (- - -). The area was calculated according to Equation 7.16 (with Model 1, i.e. assuming %T decrease is exclusively due to fouling). The volume of lysate was considered to be 100 L, to be processed in a total time of 240 minutes (including regeneration time), to a final yield of 96%.

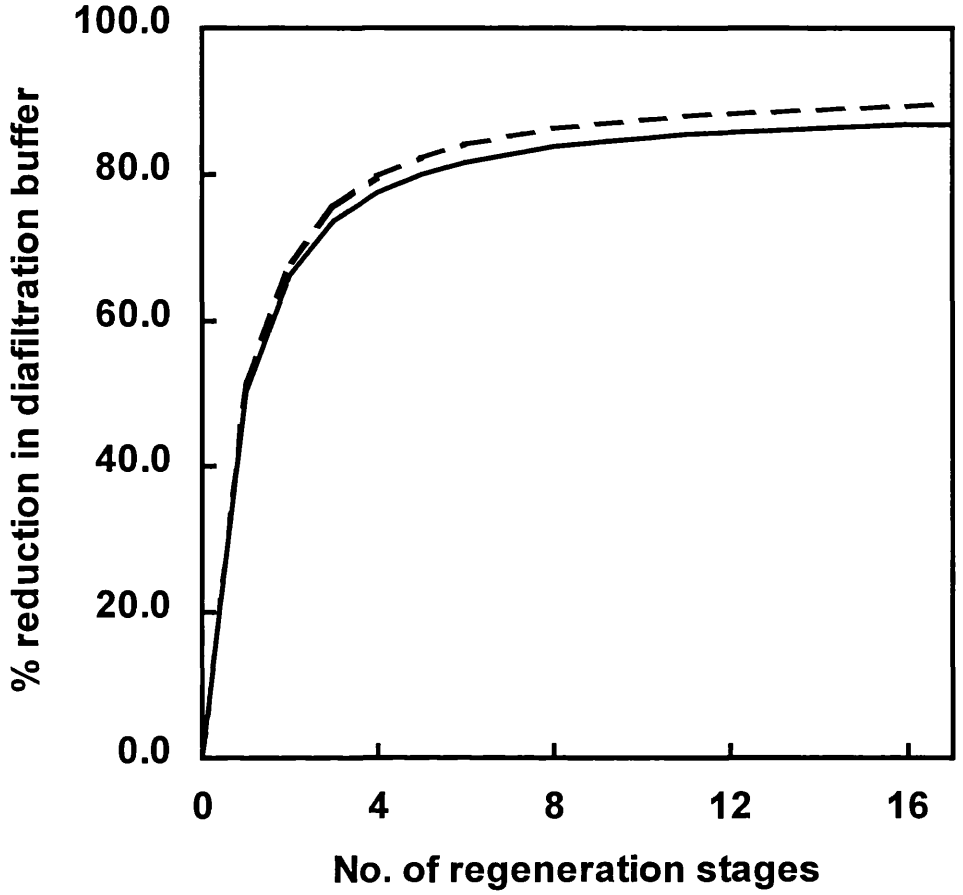


Figure 7.4 Percentage reduction in diafiltration buffer as a function of the number of regeneration stages, calculated for t=10 minutes regeneration time, both excluding the volume of buffer required for rinsing (- - -) and including the buffer required for rinsing (—). The membrane area was calculated according to Equation 7.16 (with Model 1, i.e. assuming %T decrease is exclusively due to fouling). The volume of lysate was considered to be 100 L, to be processed in a total time of 240 minutes (including regeneration time), to a final yield of 96%.

One further possibility is that transmission is not fully re-established to its initial level but only partially improved. Considering the rate of decay (a) remains unchanged, the transmission in stage n, $T^{(n)}$, can be defined by:

$$T^{(n)} = T_0^{(n)} e^{at}$$

Equation 7.18

where T_0^n is the value of transmission at the beginning of processing stage n. Also the time for each processing step will now be different, since the system loses some performance over time. So the total process time will be given by:

$$t = \sum_{n=1}^{N} t_P^{(n)} + (N-1)t_R$$

Equation 7.19

The initial value of transmission is lower than the initial value in the previous stage according to:

$$T^{(n)} = WT_0^{(n-1)} e^{at}$$

Equation 7.20

where W is the transmission recovery (0<W<1). In the previous example (Figure 7.3) W was 1 (100% recovery of transmission).

Equation 7.4 can be rewritten for step n:

$$\int_0^{t(n)} T^{(n)} dt = \frac{-V}{NJA} \ln(1-Y)$$

Equation 7.21

and for step n+1:

$$\int_0^{t(n+1)} T^{(n+1)} dt = \frac{-V}{NJA} \ln(1-Y)$$

Equation 7.22

The values of A, V (V/N) and Y are the same for each step and so the right hand side of these two equations is the same, and the left hand sides can be equalised:

$$\int_0^{t(n)} T_0^{(n)} e^{at}\,dt = \int_0^{t(n+1)} W T_0^{(n)} e^{at}\,dt$$

Equation 7.23

After integration:

$$t_P^{(n+1)} = \frac{1}{a}\ln\left(\frac{1}{W}\left(\exp(at_P^{(n)})-1\right)\right)$$

Equation 7.24

And so:

$$t = (N-1)t_R + t_P^1 + \sum_2^N\left(\frac{1}{a}\ln\left(\frac{1}{W^{n-1}}\left(\exp(at_P^{(1)})-1\right)+1\right)\right)$$

Equation 7.25

The area can be obtained by solving Equation 7.25 above to $t_P^{(1)}$ (using Solver in Microsoft Excel) and then substituting this value in Equation 7.16. Figure 7.5 shows the results for different values of W and of regeneration stages. It can be seen that even in a case where only 70% of the initial transmission is recovered it is still possible to save 30% in the membrane area required with two regeneration stages.

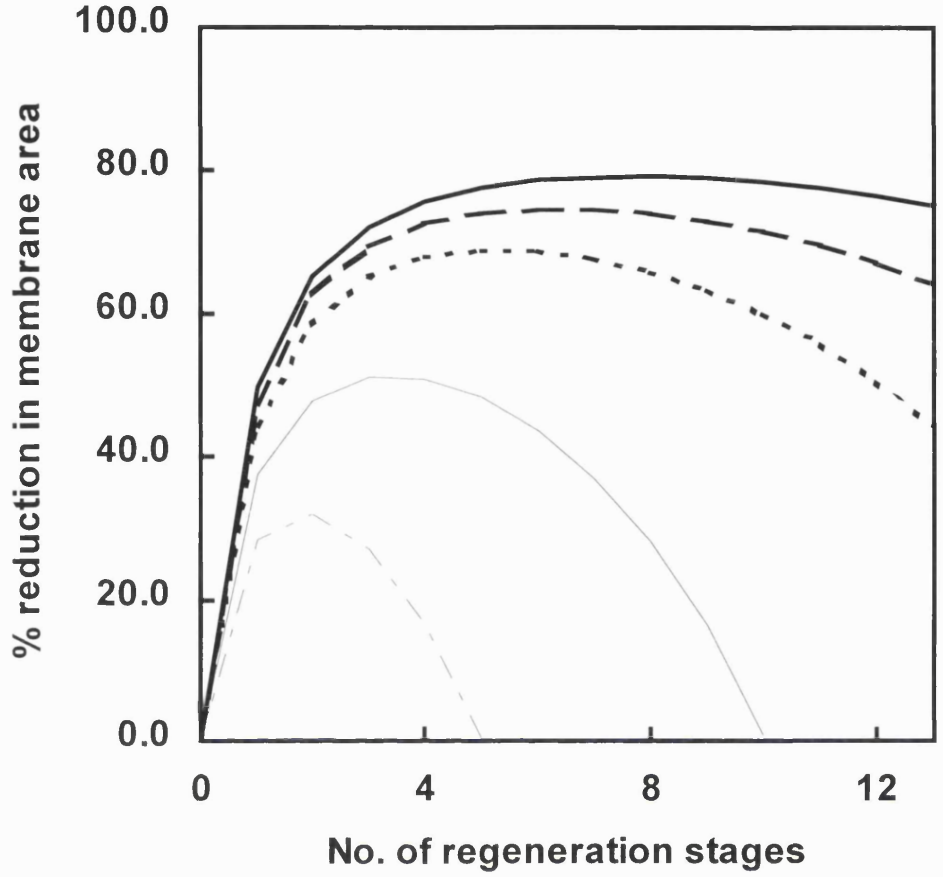


Figure 7.5 Percentage reduction in membrane area as a function of the number of regeneration stages, calculated for 10 minutes regeneration time and for different values of W (%T recovery after rinsing): W= 1 (——), W= 0.95 (— —), W= 0.9 (- - -), W= 0.8 (——) and W= 0.7 (- — -). The area was calculated according to Equation 7.16 and Equation 7.25 (with Model 1, i.e. assuming %T decrease is exclusively due to fouling). The volume of lysate was considered to be 100 L, to be processed in a total time of 240 minutes (including regeneration time) and the final yield was 96% for all cases.

7.3.3 Membrane area savings calculated with Model 2

In this case it is assumed that the level of transmission decreases with time according to Equation 7.14, which along with a %T due to fouling also assumes that a fraction of Fab' is not available for recovery. Also it is assumed that the levels of transmission can be recovered fully by a regeneration step of duration t_R, as in Equation 7.15.

The area can be obtained with the Solver tool of Microsoft Excel so that it satisfies:

$$-\frac{JA}{V}\int_0^{t'} T_{obs}\,dt = \ln(1-Y)$$

Equation 7.26

where T_{obs} is defined as in Equation 7.14.

Assuming the yield is calculated only in terms of the "available" product T_{obs} can be replaced by T_{real} (as defined in Equation 7.8) and:

$$A = \frac{-V\ln(1-Y)}{NJ\frac{T_0'}{a'}(e^{a't}-1)}, \text{ for } t=t_P$$

Equation 7.27

The variables T_0' and a' that define T_{real} were obtained from the fit of the experimental data of the diafiltration of spun down lysate (where all Fab' should be available for transmission),

Figure 7.6 shows the area savings as a function of the number of regeneration stages, for different regeneration times. The savings achievable in this example are smaller than in Figure 7.3 due to the slower %T decay, i.e. the recovery is not as spectacular. Despite this fact it is still possible to achieve 50% saving with five 5-minute rinsing steps. Lower yields were also considered to evaluate whether the local loss in yield could become economically advantageous. The membrane area saving can be increased to 70% if the yield of this step is allowed to fall down to 85%. The lower yield has obviously also negative economic implications, that will be analysed in section 7.4.

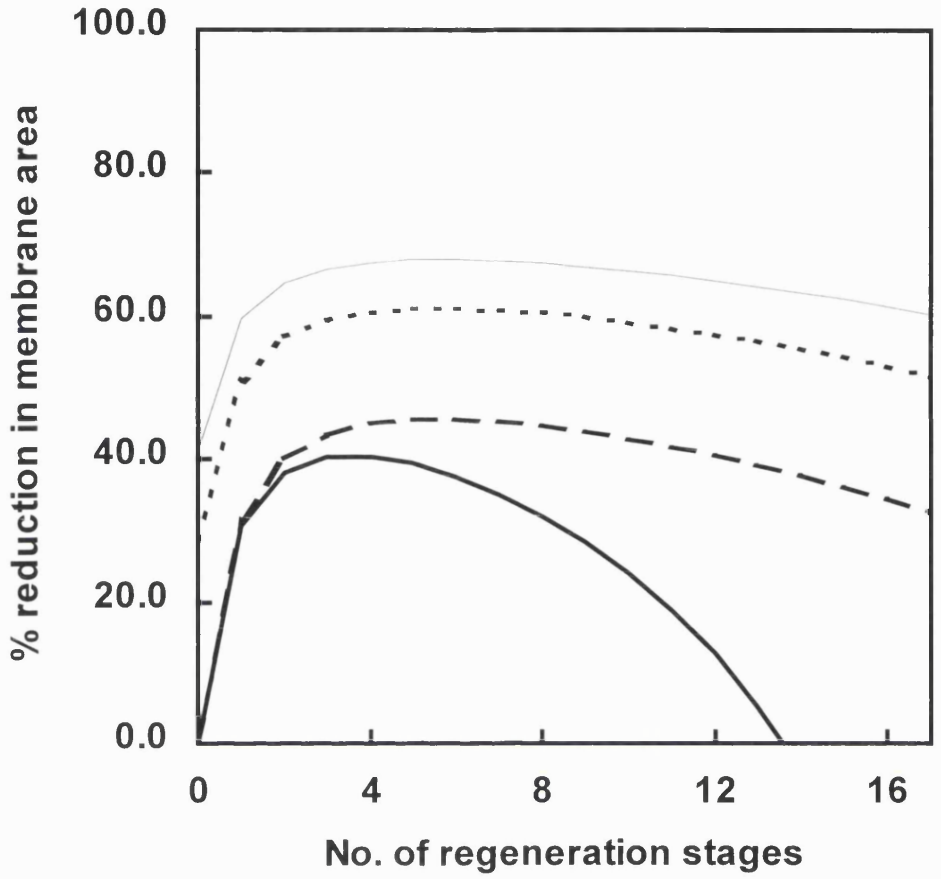


Figure 7.6 Percentage reduction in membrane area as a function of the number of regeneration stages, calculated for different yields and regeneration times: Y=0.96, t=10 minutes (——), Y=0.96, t=5 minutes (— —), Y=0.90, t=5 minutes (- - -) and Y=0.85, t=5 minutes (——). The area was calculated according to Equation 7.27 (with Model 2, i.e. assuming %T decay is due to fouling and to non-availability of a proportion of Fab'), with the final yield calculated as a function of the recoverable antibody fragment. The volume of lysate was considered to be 100 L, to be processed in a total time of 240 minutes (including regeneration time) and J=39 $Lm^{-2}hr^{-1}$.

It is also possible to investigate the impact of not recovering transmission fully with one single rinsing step. Equation 7.25 can be used with T_0' and a' instead of T_0 and a to calculate $t_P^{(1)}$, which can then be replaced into Equation 7.27 to obtain the area needed. A value of 80% recovery of transmission is very detrimental but still allows 20% saving in membrane area with 1 single 10-minute recovery step (Figure 7.7).

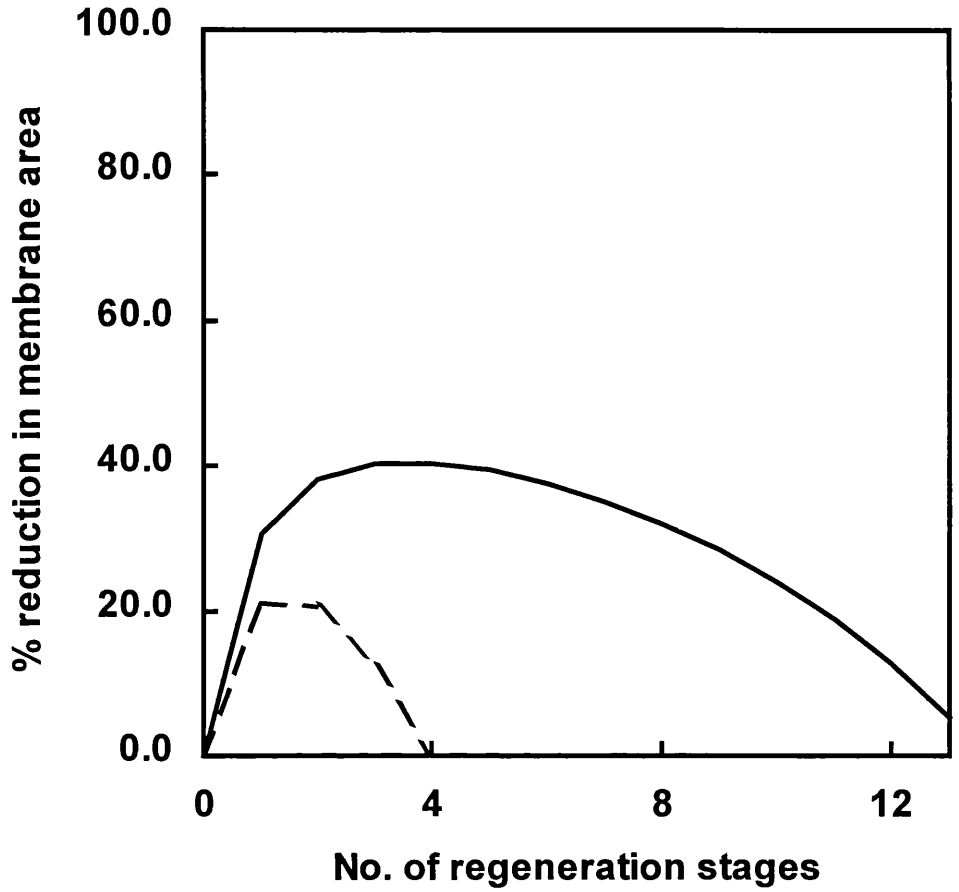


Figure 7.7 Percentage reduction in membrane area as a function of the number of regeneration stages, calculated for 96% yield and 10-minute rinsing stages for two cases: a) %T is fully recovered with the regeneration stage, i.e. W = 1 (—); b) %T is only recovered to 80% of the previous value, i.e. W = 0.8 (- - -). The area was calculated according to Equation 7.25 and Equation 7.27 (with Model 2, i.e. assuming %T decay is due to fouling and to non-availability of a proportion of Fab'), with the final yield calculated as a function of the recoverable antibody fragment. The volume of lysate was considered to be 100 L, to be processed in a total time of 240 minutes (including regeneration time) and J=39 $Lm^{-2}hr^{-1}$.

The savings in diafiltration buffer achievable are shown in Figure 7.8. In this case the volume of buffer needed for rinsing has a bigger impact due to the smaller volumes involved. The reason is that this example assumes a less fouling membrane than in the example in 7.2.2, requiring overall smaller areas.

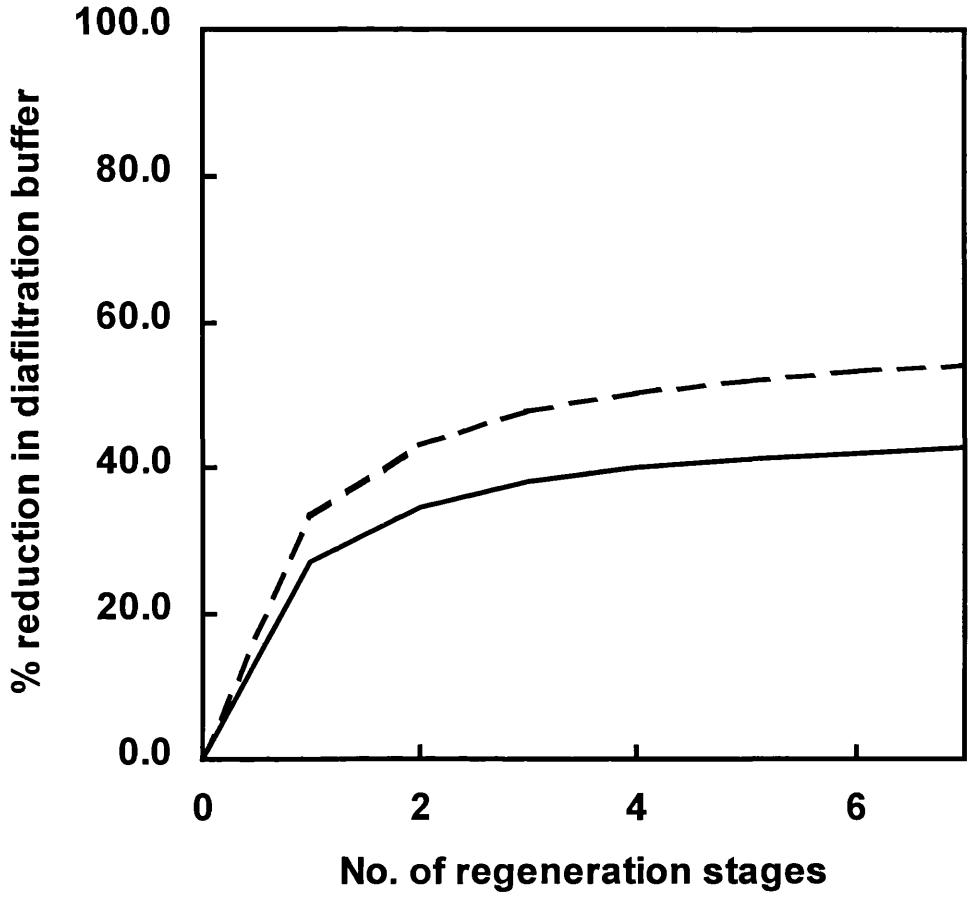


Figure 7.8 Percentage reduction in diafiltration buffer as a function of the number of regeneration stages, calculated for a yield of 96% and 10 minutes regeneration time, both excluding volume required for rinsing (- - -) and including volume required for rinsing (—). The area was calculated according to Equation 7.27 (with Model 2, i.e. assuming %T decay is due to fouling and to non-availability of a proportion of Fab'), with the final yield calculated as a function of the recoverable antibody fragment. The volume of lysate was considered to be 100 L, to be processed in a total time of 240 minutes (including regeneration time).

7.4 Economic implications

The savings in membrane area calculated above can be translated into savings in running costs through the use of the models developed in Chapter 2 and 3 and based on the case study presented in Chapter 4.

There are 3 membrane steps in the process chosen as the case study (see Figure 4.3). It will be considered as a simplification that the membrane regeneration steps are only performed for the lysate clarification step, which is the one that was thoroughly studied in the present work.

The running costs are reduced through a combination of factors. The cost of the diafiltration buffer has an almost negligible impact on the running costs, so its cost reduction will not be considered in the coming calculations for simplification purposes. The volume of diafiltration buffer required does however impact the last membrane step (UF concentration) since there will be a smaller volume to concentrate, and therefore a smaller area will be needed. This is a significant cost and for that reason the area reduction in the UF concentration step will be taken into account in the running costs calculations that follow.

Figure 7.9 shows how the membrane savings achievable with the rinsing strategy translate in terms of running costs. A 17% reduction in the running costs can be made with 7 rinsing steps, provided %T is fully recovered with the regeneration steps (area calculated with Model 1, as in Figure 7.3). A partial %T recovery (80%) still allows 12% reduction in the running costs with four 10-minute rinsing steps.

Figure 7.10 illustrates the impact on the running costs of the number of regeneration stages performed in the lysate clarification step, assuming %T decreases as described in Equation 7.27 (Model 2), with the values of a' and T_0' taken from the spun down lysate experiment (Figure 6.13). For the two cases where a lower yield is assumed for the lysate clarification step the whole process was redesigned in order to produce the same final quantity of antibody fragment. This implies that some pieces of equipment will have a higher price, thus explaining why the reduction in running costs for an 85% yield in the lysate clarification step is lower than that for 90% yield, despite an inversed trend in terms of membrane area. It can be seen that it is possible to save 13% in the running costs of the disposable plant when opting to do 5 rinsing stages in the lysate clarification step.

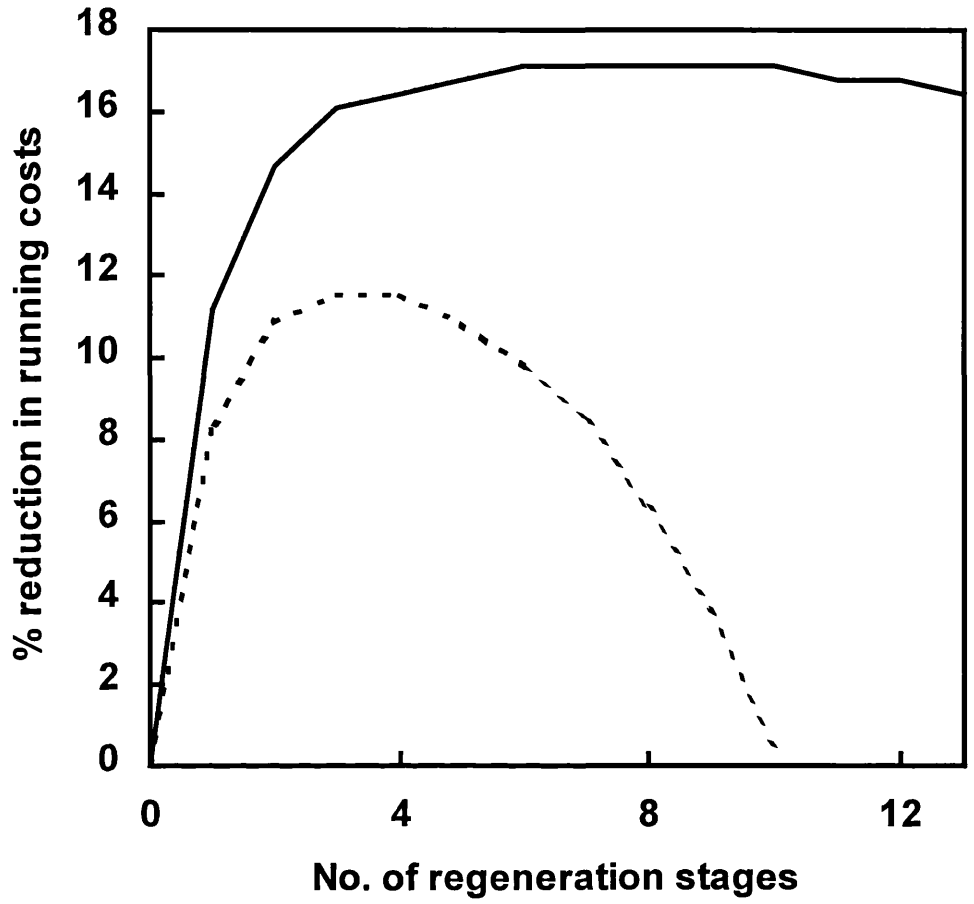


Figure 7.9 Percentage reduction in running costs as a function of the number of regeneration stages, calculated with Model 1 (i.e. assuming %T decrease is exclusively due to fouling) for 10 minutes regeneration time and two different values of W (%T recovery after rinsing) yields and regeneration times: W=1 (—) and W=0.8 (- - -). Final yield was calculated as a function of the recoverable antibody fragment. The volume of lysate was considered to be 100 L, to be processed in a total time of 240 minutes (including regeneration time) and the final yield was 96%.

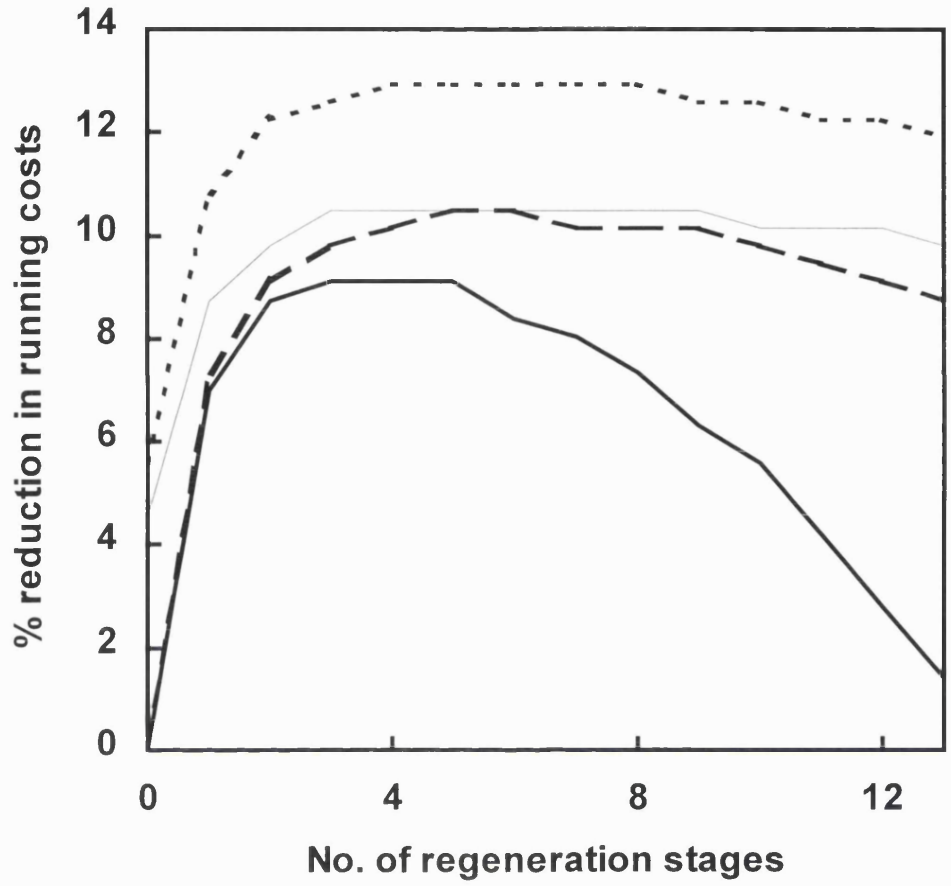


Figure 7.10 Percentage reduction in running costs as a function of the number of regeneration stages, calculated with Model 2 (i.e. assuming %T decay is due to fouling and to non-availability of a proportion of Fab') for different yields and regeneration times: Y= 0.96, t= 10 minutes (——), Y= 0.96, t= 5 minutes (— —), Y= 0.90, t= 5 minutes (- - -) and Y=0.85, t=5 minutes (—). Final yield was calculated as a function of the recoverable antibody fragment. The volume of lysate was considered to be 100 L, to be processed in a total time of 240 minutes (including regeneration time). %T is assumed to be fully recovered with each regeneration stage.

The impact of the reduction in the running costs can be translated in terms of net present value (NPV), which can be compared to the non-disposable process. NPV was calculated for the whole project, as in Chapter 4. Figure 7.11 and Figure 7.12 show the NPV ratio of the two options as a function of the number of regeneration stages. The membrane savings in the examples studied do not allow the disposable option to

become cheaper than the conventional one, since the ratio of the NPVs remains less than one. However the gap between the two options does get significantly reduced, improving from 0.75 to up to 0.9.

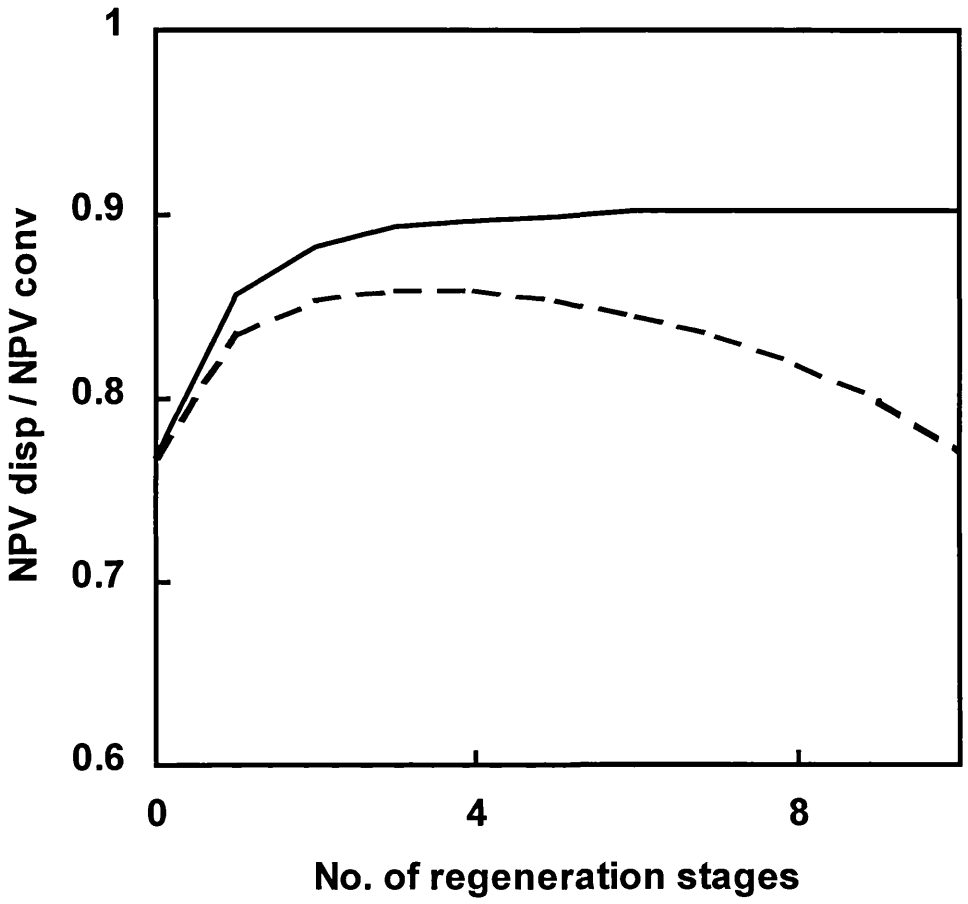


Figure 7.11 Effect on the NPV ratio of the number of regeneration stages, calculated with Model 1 (i.e. assuming %T decrease is exclusively due to fouling) for 10 minutes regeneration time and two different values of W (%T recovery after rinsing) yields and regeneration times: W=1 (—) and W=0.8 (- - -). The volume of lysate was considered to be 100 L, to be processed in a total time of 240 minutes (including regeneration time) and the final yield was 96%.

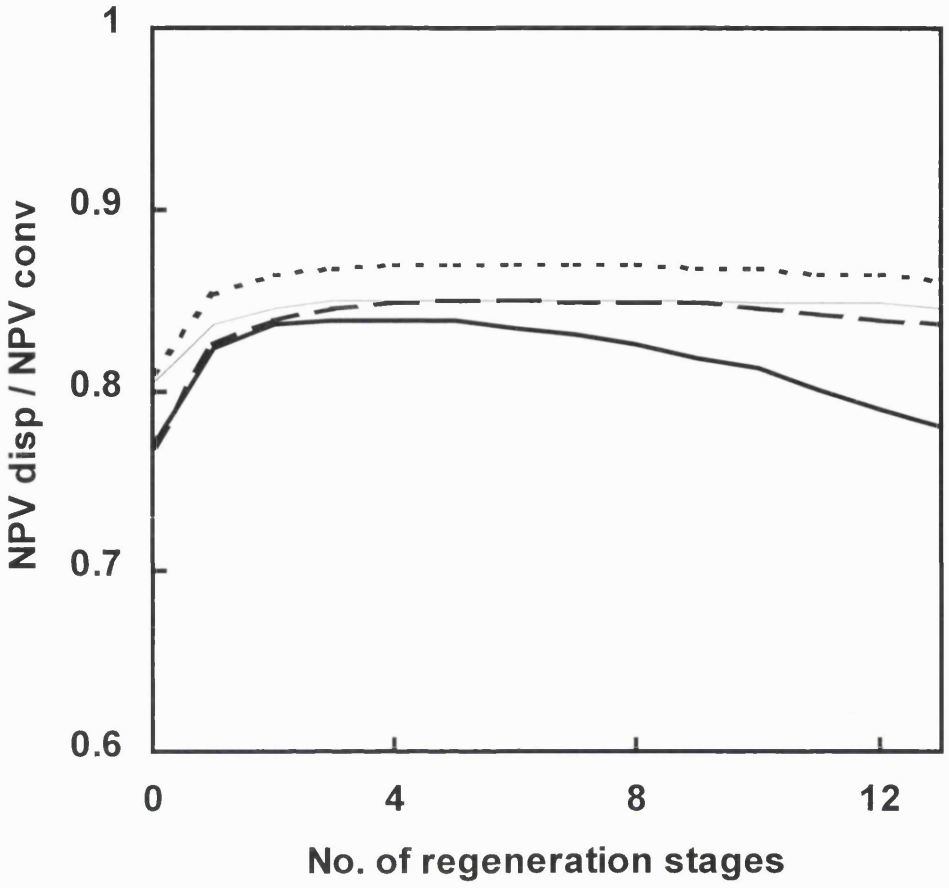


Figure 7.12 Effect on the NPV ratio of the number of regeneration stages, calculated with Model 2 (i.e. assuming %T decay is due to fouling and to non-availability of a proportion of Fab') for different yields and regeneration times: Y=0.96, t=10 minutes (——), Y=0.96, t=5 minutes (— —), Y=0.90, t=5 minutes (- - -) and Y=0.85, t=5 minutes (——). Final yield was calculated as a function of the recoverable antibody fragment. The volume of lysate was considered to be 100 L, to be processed in a total time of 240 minutes (including regeneration time).

7.5 Conclusions

In this chapter two models were studied to represent the behaviour of the transmission of a product through a microfiltration membrane. Model 1 assumed that the steep decay of transmission observed in Chapter 6 was entirely due to fouling. Although this is not an unlikely scenario, it is expected that an optimised process will at least show a slower decay. Model 2 assumed that the observed decline in transmission was not representative of the system behaviour and that the actual decrease of transmission will effectively be less accentuated, with a more moderate effect of fouling (T_{real} in Figure 7.2). According to this model the rapid apparent decay of transmission is due to approximately 20% of the initial Fab' not being "available" for the separation.

Both models were used to predict the savings in membrane area attainable with an aliquoting and rinsing strategy. This approach could provide an answer to the problems of high membrane cost in disposables-based processes, since it avoids the introduction of chemical agents in the system necessary for the regeneration of the membrane. It also does not incur additional process time nor does it add to the plant downtime. Significant membrane savings can potentially be achieved with this technique, even if the recovery is only partial, i.e. in cases where some of the fouling is reversible. This translates into savings in the running costs of the disposable plant.

Additionally the results presented in this chapter were based on the system studied in Chapter 6 but may also be applied to any other biological system or separation where fouling is detrimental to the performance of the process. In fact, as long as a model is known that describes %T behaviour with time it is possible to apply the equations developed in this chapter to calculate the membrane area (Equation 7.16, Equation 7.25 and Equation 7.27).

The same aliquoting and rinsing technique can potentially be applied to recover flux in processes where the permeate pump is not used, for example in cell harvesting. The economic consequences will be similar and, in the case of the case study presented in Chapter 4, add further to the decrease in running costs. This signifies that the economic viability of disposables-based bioprocessing can be further increased.

Chapter 8 will present the experimental results of the practical application of the rinsing strategy developed in this chapter.

Chapter 8 Effect of rinsing

8.1 Introduction

In Chapter 6 %T was shown to decrease quickly during processing, a factor which was considered very detrimental in a disposables-based process. As a consequence theoretical prediction of the effect of rinsing designed to improve the overall productivity of the process was carried out in Chapter 7. In the present chapter experiments were performed to test the hypotheses that these models generated. The experiments were designed to investigate further the causes for %T decay and whether it can be overcome by the application of intermediate rinsing steps. The direct consequences of an improved transmission would be an increase of the overall productivity of the process, ultimately resulting in a reduction of the required membrane area and hence running costs.

8.2 Results and discussion

8.2.1 Process resumed with fresh lysate after rinsing

8.2.1.1 Ten-minute rinse

Hitherto membrane rinsing has been investigated only as a method for reducing overall consumption of cleaning agents (Nakanishi and Kessler, 1985 ; Cabero et al., 1999). In the present work the use of intermittent rinsing with diafiltration buffer will be examined as a means to maintain high transmission during the process.

Experiments were conducted in which the process was interrupted before the percentage transmission had dropped below 20%, i.e. after 20 minutes. The performance of the membrane was then restored by rinsing before proceeding with the clarification step. This was achieved by replacing the lysate solution with diafiltration buffer recirculated at the same crossflow as the process and at zero transmembrane pressure (no permeate flow). Use of diafiltration buffer has the advantage of not introducing any foreign cleaning agents into the system, as well as preserving the same osmotic pressure in the spheroplasts left in the system. The percentage transmission could be restored to its original level with a 10 minutes rinsing step (Figure 8.1) with a similar rate of decline (similar first order rate constant).

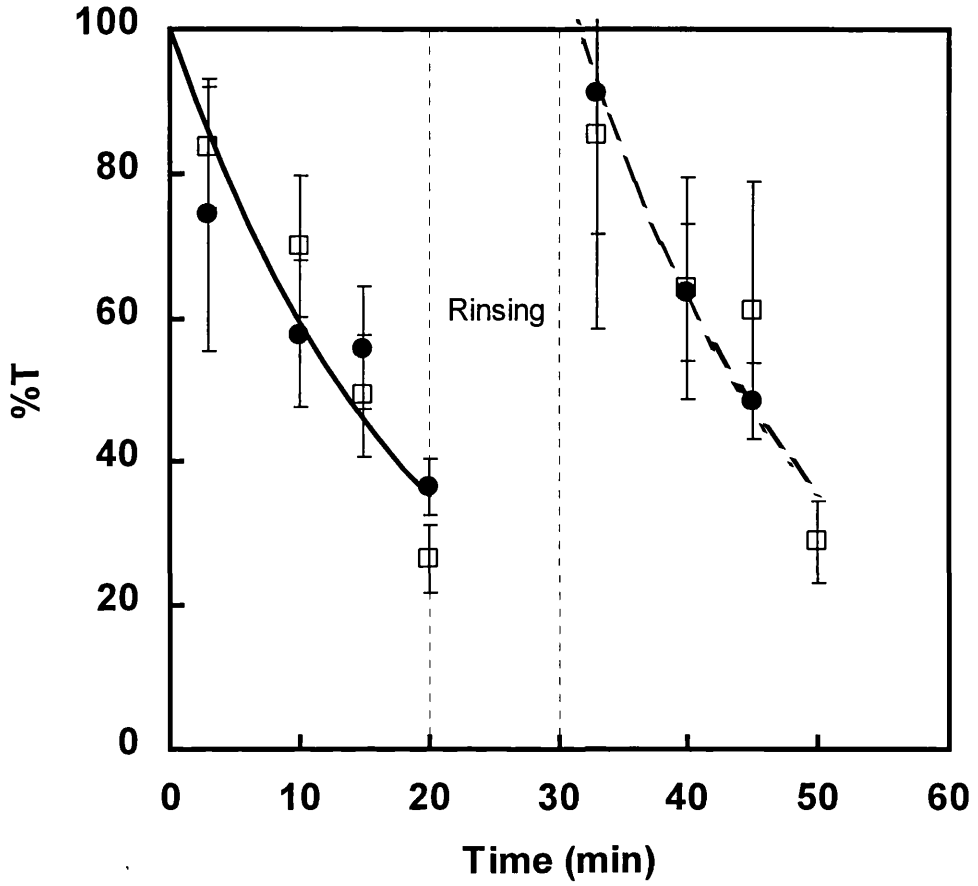


Figure 8.1 Effect of a 10-minute rinsing stage during diafiltration on the recovery of percentage transmission of antibody fragment, process resumed with fresh lysate. Rinsing was conducted at zero transmembrane pressure and at same constant retentate flow rate with 0.5 L of diafiltration buffer (150 mM NaCl). The symbols ● and □ represent two repeats of the experiment. Error bars are the propagated error from the standard deviation as a result of two to four dilutions of each permeate and retentate sample. An exponential decay fit is assumed for both stages, obtained from both repeats ($\%T = 99e^{-0.052t}$, $r^2 = 0.83$ for before rinsing and $\%T = 110e^{-0.056t'}$ ($t' = 0$ when $t = 30$ minutes), $r^2 = 0.87$ for after rinsing). Both process stages were conducted under constant cell concentration (47 g dcw L^{-1}) and constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). TMP varied between 0.14 and 0.17 bar in experiment ● and between 0.14 and 0.21 bar in experiment □. The permeation flux was maintained at 37.5 $Lm^{-2}h^{-1}$. The volume of lysate was 0.5 L for both stages.

8.2.1.2 One-minute rinse

Considering the rinsing step corresponds to downtime in terms of productivity, it is important to attempt to reduce the regeneration time to a minimum. For this reason experiments were made where the recovery step was reduced to 1 minute (Figure 8.2). As was observed for the 10-minute rinse experiment (Figure 8.1) there is no noticeable difference between the two stages of filtration in terms of %T. It can therefore be concluded that if rinsing does contribute to the recovery of %T, 1 minute is enough to realise the full benefits. In the remainder of this chapter 1 and 10-minute recovery steps will be used interchangeably.

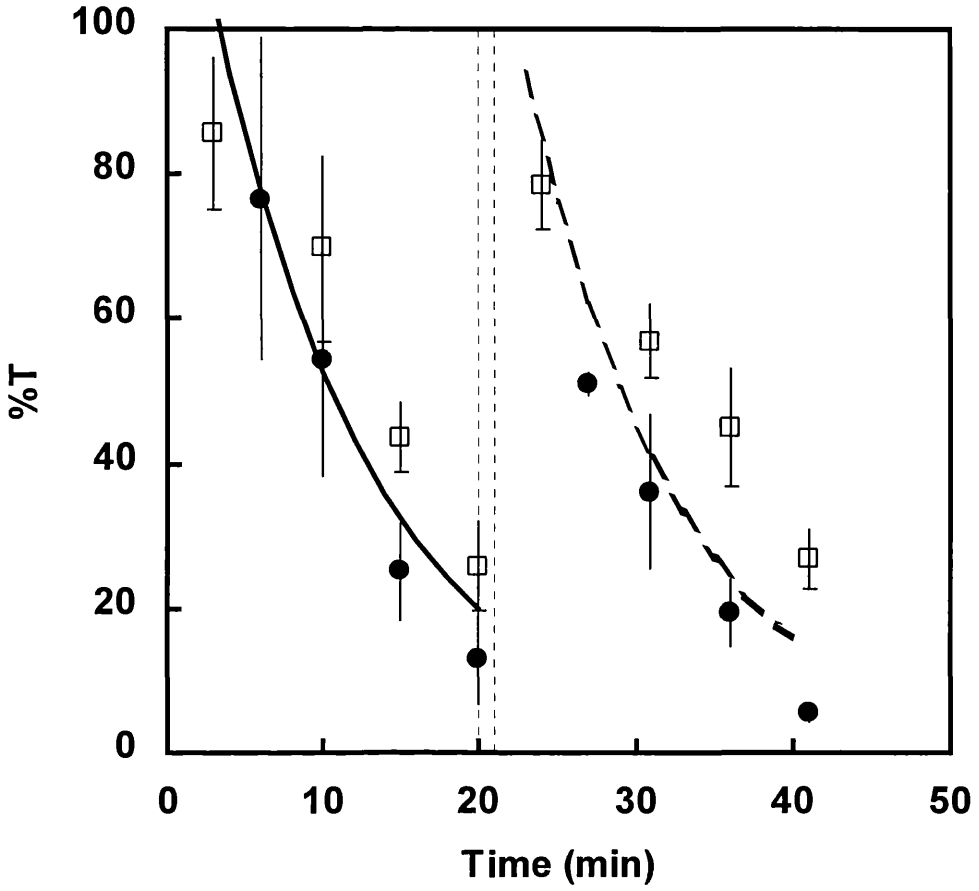


Figure 8.2 Effect of interrupting diafiltration with a 1-minute flush on the recovery of percentage transmission of antibody fragment. The interruption was done after 20 minutes processing time before resuming the process with fresh lysate. The symbols ● and □ represent two repeats of the experiment. Error bars are the propagated error from the standard deviation as a result of two to four dilutions of each permeate and retentate sample. An exponential decay fit is assumed for both stages, obtained from both experiments: $\%T=139e^{-0.097t}$, $r^2 = 0.83$ *and* $\%T=116e^{-0.104t'}$ *(t' taken as 0 for t=21 minutes),* $r^2 = 0.61$ *for before (----) and after interruption (- - -) respectively. Both process stages were conducted under constant cell concentration (47 g dcw* L^{-1}*) and constant retentate flow rate (average velocity over membrane 0.4 m* s^{-1}*). TMP varied between 0.14 and 0.17 bar in experiment ● and between 0.09 and 0.14 psi in experiment □. The permeation flux was 39.5* $Lm^{-2}h^{-1}$ *in both experiments.*

8.2.2 Process resumed with partially processed lysate after rinsing

However, when the process was resumed with the same partially processed lysate an apparent recovery in %T was not observed (Figure 8.3). This result confirms that the decrease in %T is related to the composition of the feed, although it is somewhat contradictory when compared with the diafiltration experiment performed with spun down lysate (Figure 6.13). In that experiment the feed composition changed at the same rate but the decay of %T was slower. The key difference may have been the absence of cell debris.

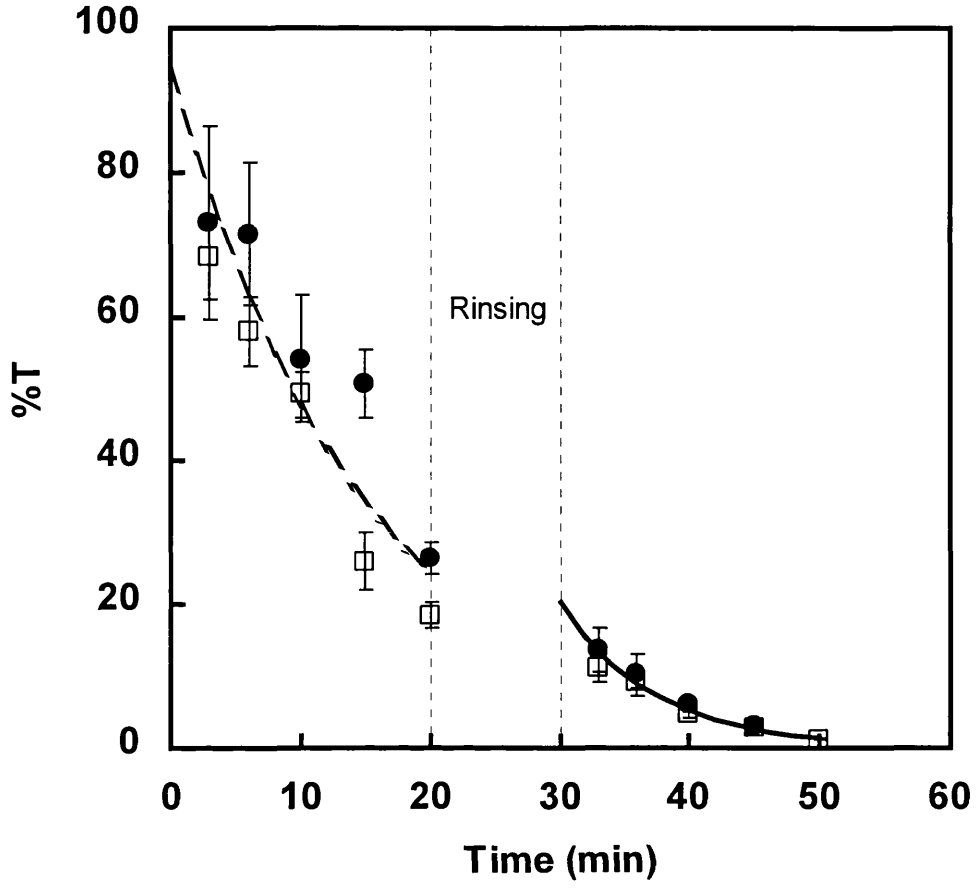


Figure 8.3 Effect of a 10-minute rinsing stage during diafiltration on the recovery of percentage transmission of antibody fragment, process resumed with partially processed lysate. Rinsing was conducted at zero transmembrane pressure and at same constant retentate flow rate with diafiltration buffer (150 mM NaCl). The symbols ● and □ represent two repeats of the experiment. Error bars are the propagated error from the standard deviation as a result of two to four dilutions of each permeate and retentate sample. An exponential decay fit is assumed for both stages, obtained from the two repeats: $\%T = 95e^{-0.068t}$, $r^2 = 0.83$ *for before rinsing (----) and* $\%T = 20e^{-0.136t'}$ *(t' = 0 when t = 30 minutes),* $r^2 = 0.97$ *for after rinsing (——). Both process stages were conducted under constant cell concentration (47 g dcw* L^{-1}*) and constant retentate flow rate (average velocity over membrane 0.4 m* s^{-1}*). TMP varied between 0.10 and 0.21 bar in both experiments. The filtration flux was maintained at 37.5* $Lm^{-2}h^{-1}$ *in both experiments. The volume of lysate was 0.5 L.*

8.2.3 Effect of an addition of concentrated permeate

Another series of experiments was conducted in which the diafiltration process was interrupted after 20 minutes and resumed after addition of 20 mL of concentrated permeate, which was obtained through 2 stages of UF (10 kD molecular weight cut-off) from an initial volume of 1310 mL. It can be seen from Figure 8.4 that the addition of concentrated permeate restored the transmission levels. At the end of the first diafiltration step the concentration of Fab' was down to 40% of the initial level (calculated based on the concentration of the retentate after 3 minutes of processing). The addition of concentrated permeate raised it back to 80% of the initial value and, interestingly, the value of %T after 3 minutes of processing in the second step is also 80% of that at the same instant of the first step. This is a further indication that %T might be related to the concentration of Fab' fragment.

The first order rate constant for the %T decay process is more negative for the second stage (following one period of rinsing), which may indicate a faster decay was occurring. On the other hand the initial value of %T was very similar for both stages, which would imply that there was a full recovery with the rinsing and the addition of concentrated permeate.

Analysis of permeate and concentrate samples by Bradford assay showed the transmission of total soluble protein follows a similar trend to that of Fab' (Figure 8.5). Additionally the ratio of Fab' antibody fragment to the total protein in the system remained unchanged throughout the process (Figure 8.6), thus showing Fab' was retained in the same way as the other proteins. The decay in %T is therefore not related to protein fractionation, which could alter specific protein-Fab' interactions.

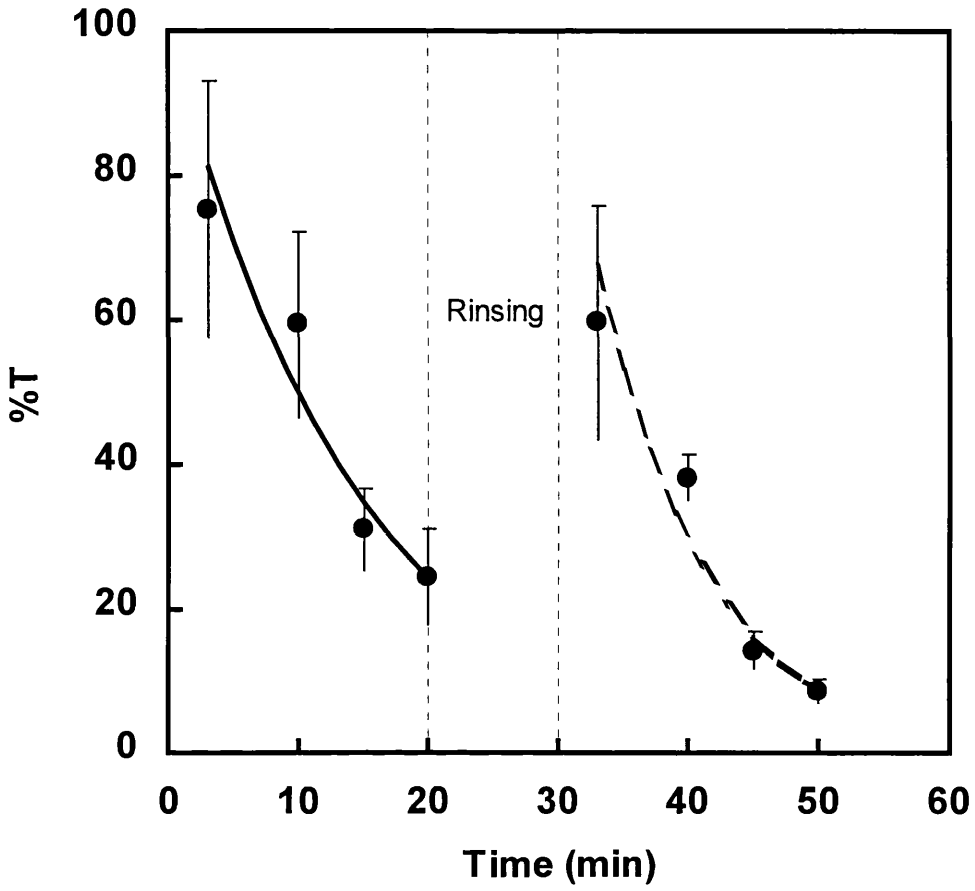


Figure 8.4 Effect on the recovery of percentage transmission of antibody fragment of an addition of concentrated permeate to the partially processed lysate, after a 10-minute rinse step. The rinsing was done after 20 minutes processing time. Error bars are the propagated error from the standard deviation as a result of two to four dilutions of each permeate and retentate sample. Exponential decay fits are assumed for each stage: $\%T = 100e^{-0.071t}$, $r^2 = 0.94$ *for before rinsing,* $\%T = 96e^{-0.119t'}$ ($t'=0$ *for* $t=30$ *min),* $r^2 = 0.96$ *for after rinsing. Both process stages were conducted under constant cell concentration (47 g dcw* L^{-1}*) and constant retentate flow rate (average velocity over membrane 0.4 m* s^{-1}*). TMP varied between 0.14 and 0.24 bar. Antibody concentration in the feed was spiked up to 80% of the initial value (based on the readings of retentate concentration at* $t=3$ *minutes and* $t=33$ *minutes).*

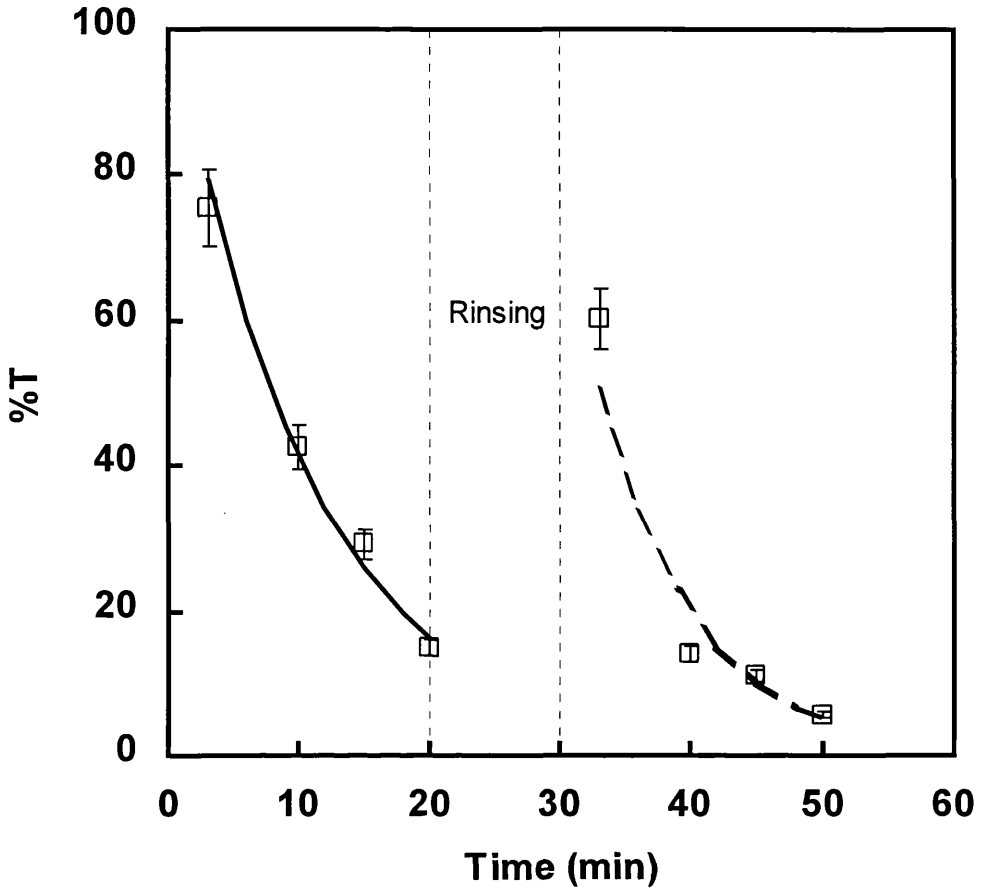


Figure 8.5 Effect on the recovery of percentage transmission of total protein of an addition of concentrated permeate to the partially processed lysate, after a 10-minute rinse step. The rinsing was done after 20 minutes processing time. Error bars are the propagated error from the protein assay error (5%) on each permeate and retentate sample. Exponential decay fits are assumed for each stage: $\%T = 105e^{-0.093t}$, $r^2 = 0.98$ *for before rinsing,* $\%T = 76e^{-0.134t'}$ *(t'=0 for t=30 min),* $r^2 = 0.95$ *for after rinsing. Both process stages were conducted under constant cell concentration (47 g dcw* L^{-1}*) and constant retentate flow rate (average velocity over membrane 0.4 m* s^{-1}*). TMP varied between 0.14 and 0.24 bar.*

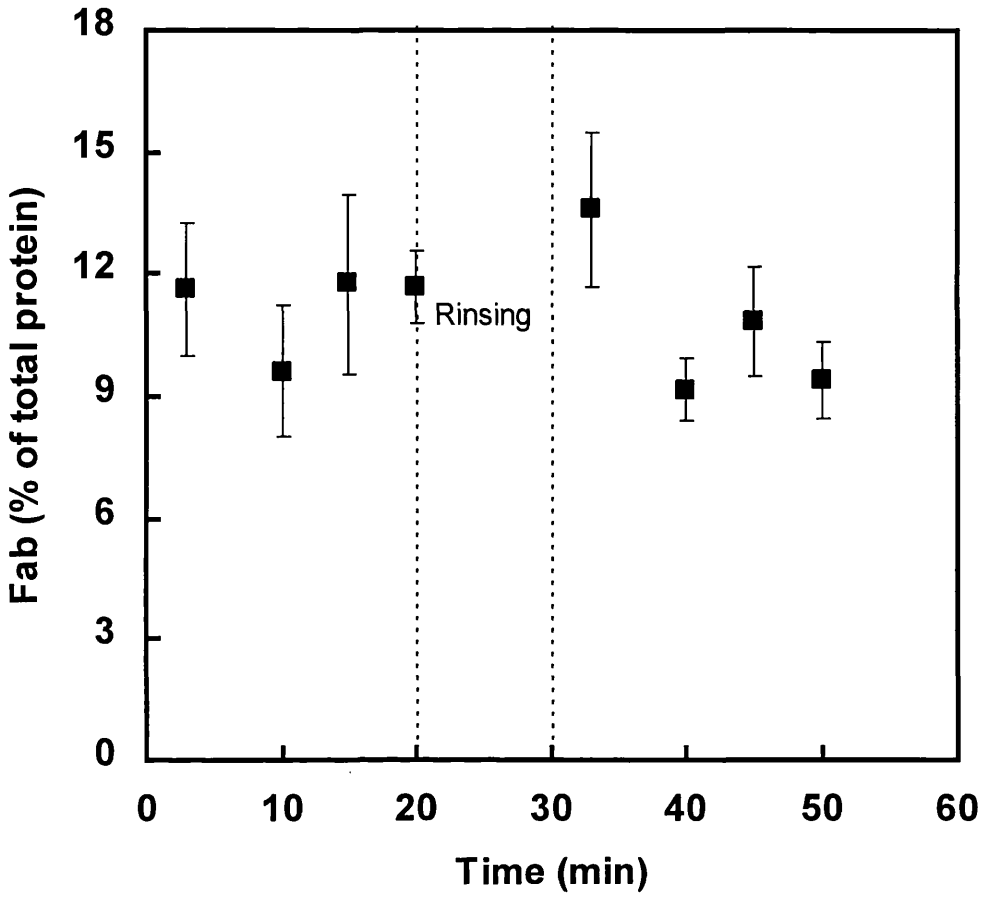


Figure 8.6 Time dependence of concentration of Fab' antibody fragment in the retentate, expressed as a % of the concentration of total protein, during diafiltration, and effect of an addition of concentrated permeate at t = 30 minutes, after a 10-minute rinsing step. Error bars are the propagated error from the standard deviation of two to four dilutions of each retentate sample (ELISA assay) and the error of the protein assay (5%). Both process stages were conducted under constant cell concentration (47 g dcw L^{-1}) and constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). TMP varied between 0.14 and 0.24 bar.

8.2.4 Effect of an addition of purified Fab'

The effect of addition of concentrated permeate was examined further. To this purpose permeate was purified with protein A affinity chromatography, as described in Chapter 5. Two fractions were obtained from this purification step: the column flow-through (i.e. all that did not bind the column) and the column eluate (purified Fab'). The addition of purified Fab' to the partially processed lysate resulted in transmission recovery (Figure 8.7) as had been observed with the addition of concentrated permeate. This is again a strong indication of a dependence of transmission on the concentration of antibody fragment. Considering there is a constant amount of Fab' in the tank that does not permeate through the membrane, this will become a less significant proportion after addition of Fab'. This hypothesis is supported by the inability to recover transmission when column flow-through (permeate without Fab') was added to the processed lysate.

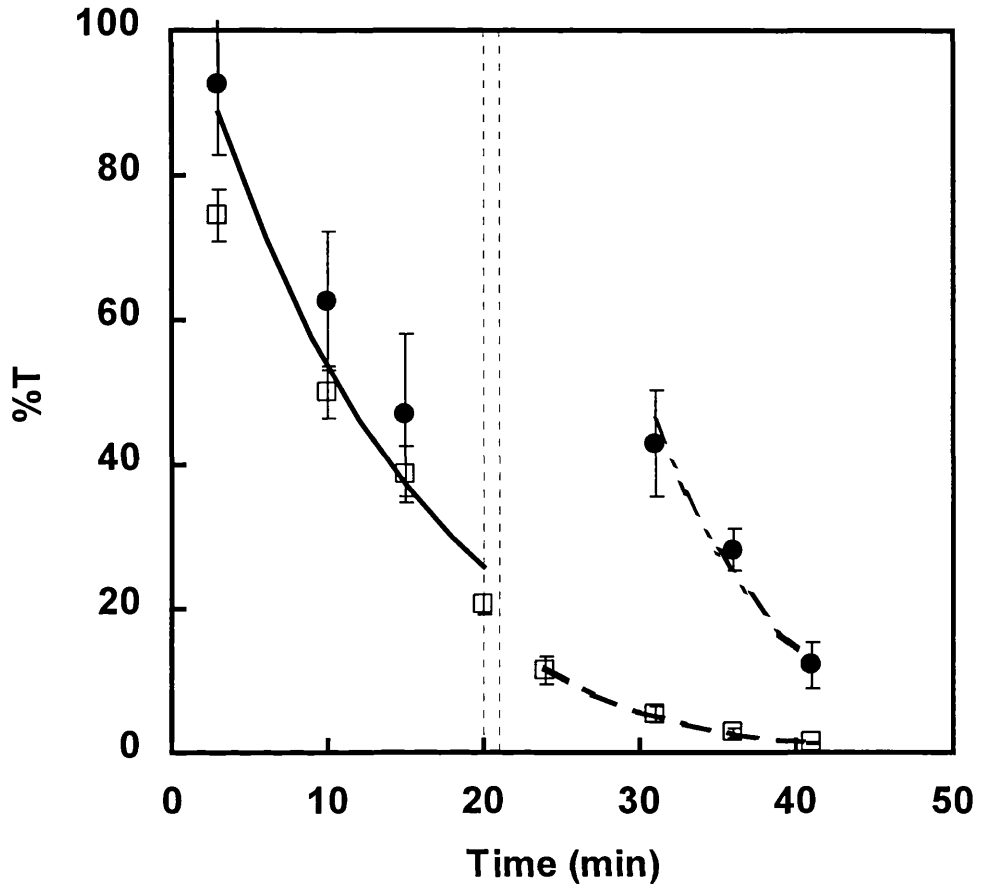


Figure 8.7 Effect on the recovery of percentage transmission of antibody fragment of an addition of concentrated Fab' (●) or concentrated column load (□) to the partially processed lysate, after a 1-minute flush interruption. The interruption was done after 20 minutes processing time. Error bars are the propagated error from the standard deviation as a result of two to four dilutions of each permeate and retentate sample. Exponential decay fits are assumed for each stage: $\%T = 110e^{-0.073t}$, $r^2 = 0.83$ *for before interruption obtained from both experiments,* $\%T = 163e^{-0.127t'}$ *(t'=0 for t=21 min),* $r^2 = 0.96$ *(●) and* $\%T = 18e^{-0.128t'}$ *(t'=0 for t=21 min),* $r^2 = 0.999$ *(□) after interruption. Both process stages were conducted under constant cell concentration (47 g dcw* L^{-1}*) and constant retentate flow rate (average velocity over membrane 0.4 m* s^{-1}*). TMP varied between 0.07 and 0.14 bar in both experiments. Antibody concentration in the feed was spiked up to 100% of the initial value (●), based on the readings of permeate concentration at t=3 and t=33 minutes.*

8.2.5 Rinsing after total permeate recycle

Assuming the diafiltration buffer has no impact on the separation performance, then total permeate recycle could be used instead of diafiltration mode to study %T behaviour. The advantage is that in total permeate recycle mode it is possible to separate the effects on %T of time and product concentration, since the latter is constant throughout the process.

For this purpose a 10-minute rinsing step was performed after processing in constant permeate recycle mode. Since the total permeate recycle experiments in Chapter 6 had indicated that after 20 minutes of processing there was no apparent fouling occurring, in the present experiment rinsing was performed after 70 minutes of processing (Figure 8.8).

The results obtained were disappointing, since after 70 minutes the levels of transmission were still at 83%, i.e. above 90% of the initial value. Additionally the rinsing step did not appear to contribute substantially, if at all, to the recovery of the transmission level. An optimistic analysis would indicate that %T increased from 83% to 85% with the rinsing step, but this level of precision is below the accuracy provided by the ELISA assay.

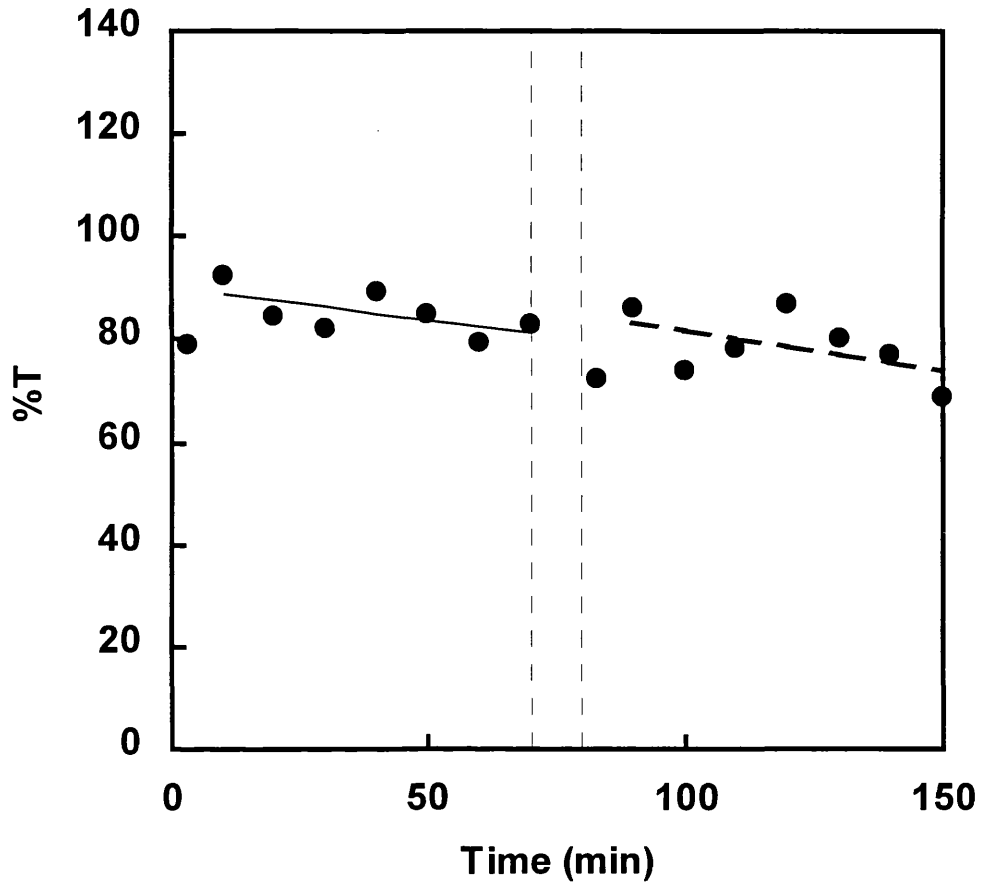


Figure 8.8 Effect of rinsing stage during total permeate recycle on the recovery of percentage transmission of antibody fragment, process resumed with fresh lysate. Rinsing was conducted at zero transmembrane pressure and at same constant retentate flow rate with 0.5 L of diafiltration buffer (150 mM NaCl). Error bars are the propagated error from the standard deviation as a result of two to four dilutions of each permeate and retentate sample. An exponential decay fit is assumed for both stages ($\%T = 90e^{-0.002t}$, $r^2 = 0.39$ for before rinsing and $\%T = 85e^{-0.002t'}$ ($t' = 0$ when $t = 80$ minutes), $r^2 = 0.27$ for after rinsing). Both process stages were conducted under constant cell concentration (47 g dcw L^{-1}) and constant retentate flow rate (average velocity over membrane 0.4 m s^{-1}). TMP varied between 0.28 and 0.36 bar in the first step and between 0.28 and 0.38 bar in the second step. The permeation flux was maintained at 36.5 $Lm^{-2}h^{-1}$ throughout both stages. The volume of lysate was 0.5 L for both stages.

8.3 Conclusions

The experimental work in this chapter was intended to be a practical application of the concepts developed in Chapter 7. The assumption was that transmission could be, at least partially, recovered with intermediate rinsing. The experimental results do show that when the process is interrupted after 20 minutes %T is fully restored to its initial level by a rinsing step of either ten or one minutes (Figure 8.1 and Figure 8.2). This indicates that if fouling has occurred it was reversible since it was removable by rinsing at zero transmembrane pressure (Shorrock and Bird, 1998). This conclusion would also be consistent with that of Crozes et al. (1997), who observed that operation at low TMP prevents irreversible fouling. Some results in Chapter 6 however indicated that the decrease in %T might not due to fouling.

During total permeate recycle experiments (Figure 6.5) there is no apparent fouling in the first 40 minutes. If total permeate recycle is representative of the diafiltration process this would mean that a rinsing step made after 20 minutes is premature. For this reason an experiment was performed where total permeate recycle was interrupted after a long period of time (70 minutes), followed by rinsing to assess the effectiveness of the recovery step (Figure 8.8 in section 8.2.5). This experiment was inconclusive in two ways. On the one hand only a little fouling occurred within 70 minutes of processing, which effectively reduces the impact of any recovery obtainable with the rinsing step. Additionally the recovery does appear to be very low. In the above it must be remembered that total permeate recycle is not a mode for product separation, and it will have to be checked that the fouling occurring in this mode is identical to that observed during actual separation, e.g. diafiltration.

This chapter also allows further substantiation of some of the conclusions made in Chapter 6. For example the recovery of transmission after addition of concentrated permeate (Figure 8.4) and indeed also of purified Fab' (Figure 8.7) indicates that, provided cell debris are present, the concentration of the product itself (and not other soluble substances present in the system) is the principal determinant of the values of transmission.

The rinsing strategy developed in Chapter 7 does not seem to apply to the present system. Nonetheless this does not invalidate the results obtained in Chapter 7, and

other biological systems should be studied from this point of view, especially when a disposables-based process is being considered.

Chapter 9 Conclusions

The fully disposable biopharmaceutical plant is a novel concept with a diversity of potential, as described in the introductory chapter of this thesis. In particular such plants are of great interest during the product development phases, by offering increased flexibility, lower capital risk and shorter down- and turnaround-times, which can result in shorter development times.

Little information has however been available to date as to the economic viability of such plants. As a consequence the objective of the first part of this thesis was to evaluate disposables-based plants from an economic standpoint. The first step was to develop a costing framework specific to conventional biopharmaceutical processes. Most of the models currently available are based on traditional chemical engineering processes. Capital investment can be calculated through different models, of varying levels of detail. The method chosen was based on a traditional chemical engineering approach, with all the cost items factored as a function of the equipment costs. The factors were adapted to reflect better biochemical engineering based on data and comments provided by the industrial partners. Running costs models showed similar levels of diversity and in this case a factored method was developed from an industrial example presented in the literature (Datar et al., 1993). This model allows the calculation of the running costs directly from the capital investment.

In Chapter 3 the economic models were adapted so as to accommodate the specific features of disposables processes. Assumptions had to be made based on the concept of disposables-based plants, as presented in Chapter 1, since actual information on such processes does not yet exist. The first key conclusion was that the capital investment required to set-up a biopharmaceutical process is reduced by 40% when a disposable approach is followed. The main contributing factors were the major reduction in equipment and pipework costs, which switch from capital costs to become running costs, as well as a reduction in building costs, validation costs, etc. The lower capital investment was counteracted to a large extent by a 70 or 90% increase in running costs, according to whether a bacterial or mammalian cell process is considered. This

higher cost is mainly due to the single-use nature of the process (i.e. the contribution of disposable equipment costs), despite the decrease in other costs such as utilities and maintenance. Significant savings in time to market (up to 1.5 years) were however predicted, as a result of shorter plant construction time, reduced down-time during product materialisation for clinical trials, etc.

The net present value (NPV) was the economic indicator used to combine capital investment and running costs and to compare the two manufacturing options. The production of a Fab' antibody fragment through an *E. coli* fermentation was chosen as representative of a generic biopharmaceutical process. Some changes in the process had to be made so as to exclude equipment that is not disposable by nature (e.g. centrifuges). The NPV of the disposables-based option was found to be positive, and hence economic viable. Additionally the disposable NPV is close to that of the conventional option (25 % lower), which together with the increased flexibility and shorter time to market, makes this an attractive alternative to conventional technology.

Sensitivity analysis showed that for the disposable case a reduction in the achievable yield in either fermentation or chromatography steps would result in a lower NPV but that this could be overcome by a reduction in disposable equipment costs.

The cost of membranes is the principal reason for the high running costs in a disposable approach, contributing 64% of the materials costs, followed to a much lesser extent by the cost of the chromatography matrices (18% of the materials costs). The impact of these costs on the economics of disposables-based plants can be tackled in two different ways:

- Membranes and columns can be replaced by modules designed specifically for single-use and consequently of cheaper construction

- Different operation strategies can be developed that will allow an improvement of the performance of these unit operations, which ultimately results in reduced membrane areas or column volumes.

The first strategy was examined through a sensitivity analysis in Chapter 4, which indicated that substantial savings can indeed be achieved with disposable modules,

cancelling out the cost gap between the two options. The second strategy constituted the basis of the second part of the thesis, focused exclusively on membrane area reduction, as this represented a significantly higher cost than that of the chromatography matrices.

The membrane optimisation work was targeted at the lysate clarification step, as this was identified as the most difficult membrane separation in the case study process, thus offering more development potential. Indeed it was found that the transmission of product through the membrane during lysate clarification displayed a very sharp decline with time. This meant that the process was operated at very low performance levels for the majority of the process time. The direct consequence of the low performance was that large membrane areas would be needed for a reasonable separation time to be achieved.

Experimental work indicated that fouling was likely to be only partially responsible for the decline in transmission. Indeed an important finding made in Chapter 6 was that "non-available" product (in this study up to 20% of the initial total value) may be responsible for the decline of the observed transmission. Product aggregation or product swelling due to charge effects constitute possible reasons why some product may not be available for the separation. This result is particularly relevant because the feed material used in the present work was a real process stream, as opposed to pure protein solutions, which comprise the basis for most studies found in the literature. Also the finding may provide a reason for apparent poor performance observed in other systems.

Two models were developed to represent transmission (%T) decline as: (1) a function of time only (Model 1); a function of time and product concentration (Model 2). The significance of the latter model is that it incorporated the impact of the "non-available" product. It was also assumed that at least part of the fouling occurring in the system would be reversible, i.e. that the levels of %T can be fully or at least partially recovered through rinsing of the membrane. This strategy was shown potentially to allow for significant membrane savings that ultimately result in reduced running costs for the disposable option. As a result the NPV of the disposable option could be increased to a level equivalent to 90% of that of the conventional option.

Experiments were then performed to confirm the modelling results and the rinsing strategy with lysate of the case study process. More evidence was found to indicate the presence of "non-available" product for transmission. The rinsing strategy was shown to allow a recovery in transmission, although it was not clear if this was due to a reduction of the fouling on the membrane surface. To clarify this an experiment was performed in total permeate recycle mode, indicating that the decrease in %T due to fouling was not very important. For that reason it was hard to be conclusive about the effectiveness of the rinsing step, although it appeared to improve %T to some extent.

Overall this thesis has demonstrated that disposables-based processes constitute an attractive and economically viable alternative to conventional stainless-steel processes. Additionally the economic competitiveness of disposables-based processes could be further improved through the development of new engineering strategies for the operation of membrane separations, which lead to savings in filtration area whilst achieving acceptable levels of transmission.

Chapter 10 Future work

The review of economic models for bioprocesses (Chapter 2) revealed that little information is currently available for the costing of such processes. Future work should attempt at overcoming this through collection of real economic data from a large sample of biopharmaceutical companies. This is a difficult enterprise that will require cooperation from industry. If successful it could help validate and improve the costing methods used in this thesis.

Disposables-based bioprocessing was shown to be economically competitive with conventional methods when used at the commercial stage of manufacture. The comparison should now be extended so as to evaluate the economic impact of the use of disposables in all of the product development stages. Additionally the economic evaluation required a number of assumptions to be made as to the features of a disposables-based plant. These will have to be confirmed. For example it was assumed that some of the instrumentation could be redesigned to be non-invasive or disposable. Research work will have to be carried out in order to identify solutions to these engineering challenges.

An interesting point that can be investigated further is upon the number of batches achieved per year in a disposable plant can be improved, since there is no downtime for CIP and SIP. This would result in increased annual productivity if at a commercial stage, or a higher throughput of drug candidates if applied at the product development stages.

The transmission behaviour observed during diafiltration of *E. coli* lysate was not fully attributable to fouling, as it was not observed in total permeate recycle mode. Experiments performed on concentration mode may shed more light into the mechanisms responsible for the loss in performance. Additionally diafiltration experiments at the iso-electric point of the Fab' antibody fragment and at increasing values of ionic strength should be performed to isolate any charge effect. Such an interaction would alter the size of the antibody fragment and hence %T. Also the

presence of Fab' or Fab'/cell debris aggregates should be assessed, possibly with the use of size exclusion chromatography.

Further work will also have to be performed to confirm the rinsing strategy proposed in Chapter 7. For this purpose a range of different feed streams will have to be used. Also the rinsing strategy can potentially be extended to processes that are not operated under flux control mode, such as the cell harvesting step. In such cases the rinsing would not only serve to improve product transmission but it would also result in higher overall fluxes, i.e. increased productivity.

Appendix 1 Mass balances

	FINAL
Cells	11.4 kg
Fab' (intracellular)	180 g
Fab' (extracellular)	30 g
Volume	300 L

Table A1.1 Fermentation final balance.

	IN		OUT	
	FROM FERMENTER	**DIAFILTRATION BUFFER**	**PERMEATE (discarded)**	**RETENTATE**
Cells	11.4 kg	0	0	11.4 kg
Fab' (intracellular)	180 g	0	0	180 g
Fab' (extracellular)	30 g	0	27.2 g	2.8 g
Volume	300 L	76 L	300 L	76 L

Table A1.2 Cell harvesting (MF) mass balance. Note: concentration step to a final cell concentration of 150 gL^{-1} followed by 1 volume diafiltration. 3 hours process time, %T (cells)=0%, J=25 $Lm^{-2}h^{-1}$, membrane area A=3.4 m^2.

	IN		OUT
	FROM CELL HARVEST	**LYSIS BUFFER**	**FINAL**
Cells	11.4 kg	0	11.4 kg
Fab' (intracellular)	180 g	0	27 g
Fab' (extracellular)	2.8 g	0	155.8 g
Volume	76 L	76 L	152 L

Table A1.3 Periplasmic release mass balance. Note: assuming yield=85%.

	IN		OUT	
	FROM LYSIS	**DIAFILTRATION BUFFER**	**PERMEATE**	**RETENTATE (discarded)**
Cells	11.4 kg	0	0	11.4 kg
Fab' (intracellular)	27 g	0	0	27 g
Fab' (extracellular)	155.8 g	0	154.2 g	1.6 g
Volume	152 L	98.5 L	220.1 L	30.4 L

Table A1.4 Lysate clarification (MF) mass balance. Note: 5-fold concentration step followed by 3.2 volumes diafiltration. 4 hours process time, %T (Fab) =95%, J=10 $Lm^{-2}h^{-1}$, membrane area A=5.88 m^2, Yield=99%.

	IN		OUT	
	FROM CLARIFICATION	**DIAFILTRATION BUFFER**	**PERMEATE (discarded)**	**RETENTATE**
Fab'	154.2 g	0	4.1 g	150.1 g
Volume	220.1 L	61.0 L	237.1 L	44.0 L

Table A1.5 Product concentration (UF) mass balance. Note: 5-fold concentration step followed by 1.4 volumes diafiltration. 2 hours process time, %T (Fab) =1% J=50 $Lm^{-2}h^{-1}$, membrane area A=2.79 m^2.

	IN			OUT	
	FROM UF	**WASH BUFFER (A)**	**ELUTION BUFFER (B)**	**WASTE**	**PURIFIED FAB**
Fab'	150.1 g	0	0	7.5 g	142.6 g
Volume	44.0 L	75.1 L	22.5 L	119.1 L	22.5 L

Table A1.6 Affinity chromatography mass balance. Note: Column capacity: 20 gL^{-1}, yield: 95%. The separation also required 61 L glycine 1.2 M (added to the feed before loading into the column) and 7.5 L 6 M guanidine for column wash.

Appendix 2 Equipment costs

ID	Description	Working Volume (WV)	Suggested WV	Budget Price (£ 000s)
F1	Seed fermenter	30L	-	70
F2	Fermenter	300L	-	196
T1	Media preparation tank	30L	100L	23
T2	Media preparation tank	300L	300L	27
T3	Buffer preparation tank	76L	100L	23
T4	Agitated Tank	300L	300L	27
M1	MF module	3.36m2	-	20
T5	Agitated Tank	152L	300L	27
T6	Buffer preparation tank	76L	100L	23
T7	Buffer preparation tank	98.5L	100L	23
U1	UF module & Controller	5.88m2	-	38
U2	UF module & controller	2.79m2	-	38
T8	Agitated Tank	221L	300L	27
C1	Chrom. column & Controller	7.5L	-	60
T9	Buffer preparation tank	7.5L	Bottle / Bag	0
T10	Buffer preparation tank	75L	100L	23
T11	Buffer preparation tank	23L	100L	23
T12	Agitated Tank	25L	100L	23
P	Vessel Panels (11 off)	£2,500 each	-	27.5
	Total			**£718,500**

Table A2.1 Fixed equipment inventory and correspondent budget costings (Doyle, 1999). See Figure A2.1 for process diagram and equipment ID.

Utility Type	Description	Comments	Budget (£ 000s)
Developed:	Compressed Air	Process & Instrument	130
	WFI Package	Generator, Tank	180
	Purified Water Package		160
	Clean Steam Package		140
	Chilled Water		70
	Glycol Water		50
	Kill Tank System	Two tank system	100
Main:	General Effluent	Collection /Treatment	25
	Natural Gas	In Building/HVAC Cost	0
	Plant Steam	In Building/HVAC Cost	0
	Fire Water	In Building/HVAC Cost	0
	Electricity	In Building/HVAC Cost	0
	Drainage	In Building/HVAC Cost	0
	Mains water	In Building/HVAC Cost	0
	Total		**£855,000**

Table A2.2 Utilities equipment list and some costs (excluding piping) (Doyle, 1999).

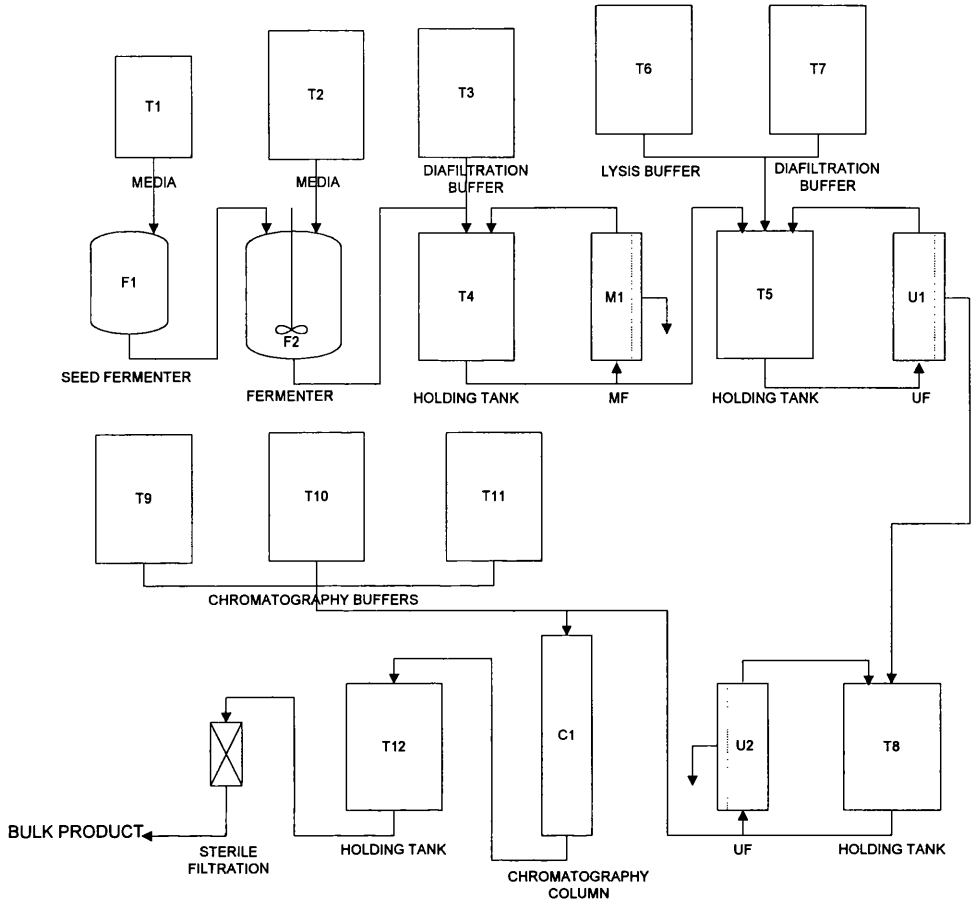


Figure A2.1 Process diagram and equipment ID.

ID	Description	Working Volume (WV)	Budget Price (£)
F1	Bag holder	30L	45
F2	Bag holder	300L	800
T1	Bag holder	30L	45
T2	Bag holder	300L	800
T3	Bag holder	76L	50
T4	Bag holder	300L	800
T5	Bag holder	152L	55
T6	Bag holder	76L	50
T7	Bag holder	98.5L	50
T8	Bag holder	221L	800
T9	Bag holder	7.5L	7
T10	Bag holder	75L	50
T11	Bag holder	23L	45
T12	Bag holder	25L	45
Total			**£3,642**

Table A2.3 Fixed equipment in disposables plant and correspondent costs (HyClone Europe price list, 1998). See Figure A2.1 for process diagram and equipment ID.

Appendix 3 Commercial appraisal of disposables-based technology

The following executive summary was written as part of the New Venture Development course in London Business School, from January to March 2001, with the collaboration of five MBA students (Francis McCullough, Scott McKinnon, Rob Schult, Sergio Sperat and Gavin Watson). The business plan evaluated the potential commercial exploitation of the disposables concept.

EXECUTIVE SUMMARY

Flex BioPharma will increase the marketable lifetime of patented biopharmaceuticals by bringing products to market more quickly using disposable manufacturing techniques. The combination of lower capital investment and faster time to market can improve the NPV return on R&D investment for a typical drug portfolio by 11%.

The Problem

The already increasing number of biopharmaceutical drugs in the development pipeline is likely to experience further dramatic growth as a result of the recent decoding of the human genome. Despite this favourable environment, biopharmaceutical research firms still face many difficulties, namely:

- The drug development and testing process currently takes an average of 6 to 8 years, significantly cutting into the 20 year patented lifetime;
- The likelihood of failing at one of the regulatory stages (for instance because of adverse side-effects) is very high. Less than 1 in 20 drugs entering clinical evaluation reaches the market;
- Biopharmaceutical firms face the dilemma of whether to build their own manufacturing facility or to outsource production to a contract manufacturer. The in-house production of biopharmaceutical drugs is an intricate process requiring major capital outlays to purchase the numerous pieces of complex equipment required. The use of contract manufacturers increases the risk of being subjected to long delays due to lack of capacity.

The Solution

Flex BioPharma offers a third production option – **fully disposable manufacturing processes**. This solution provides a cost effective resolution to the manufacturing dilemma by achieving financially significant time to market benefits for a reduced capital outlay.

Flex BioPharma is able to design and construct biopharmaceutical production lines based on single-use equipment. The concept is uncomplicated, comprising a simple clean-room outer shell with the disposable pieces of equipment and connecting tubing being brought in as needed. Advantages to the biopharmaceutical firm are as follows:

- The initial capital outlay to develop a manufacturing facility is reduced by 50%. For example, there is no need for expensive stainless steel equipment or cleaning/sterilisation capabilities;
- Pre-sterilised disposable equipment translates into significantly reduced periods of down-time between production batches, thus decreasing time to market;
- The single-use concept is inherently flexible whereas traditional, fixed, stainless steel manufacturing equipment is not only difficult to exchange but also requires additional capital commitment.

It is estimated that a disposable manufacturing plant will reduce drug development time by at least 4 to 6 months in comparison with a traditional manufacturing process. Although this is only a small fraction of the 6 to 8 year total development time, even this small change equates to **$17 million in additional revenues for a typical drug** worth $50 million annually.

Target Market

Flex BioPharma's solution is particularly attractive to SME biopharmaceutical firms as a result of the lower capital investment requirement and the greater control the firm will have over the development manufacturing process – a luxury normally only available to large and cash rich pharmaceutical companies. As a result Flex BioPharma will initially target the increasing numbers of these smaller firms who have yet to invest heavily in traditional manufacturing plants. An additional benefit of this approach to Flex BioPharma is the relatively low attractiveness of this market to the existing contract manufacturers who are expected to be our principal competitors. We believe that Flex BioPharma will be able to target the SME market, prove the capabilities of disposable technology and build up a reputation without exacting a fierce competitive reaction. Furthermore, our three years of expertise in disposable technology has culminated in a patent pending for a disposable fermentation unit, a very difficult step and a necessary part of all biopharmaceutical processes. Both our patent pending and know-how constitute valuable barriers to entry.

The time to market advantages of disposable manufacturing are equally applicable to larger biopharmaceutical operations. Therefore, we believe that once the technology has been proven contract manufacturers and Big Pharma will show great interest and fuelling the demand for our disposable technologies.

Size, Value and Growth

The biopharmaceutical industry is large, with estimated world-wide revenues of $16 billion in 1996 rising to a projected $24 billion in 2000. The US biotech industry constitutes 60% of the world's total, with Europe providing 30% and Japan 7%. The industry is expected to grow at a compound annual growth rate of between some 20% over the next 3 to 5 years.

Demographic trends such as population ageing, increased demand for life-style drugs and increased levels of wealth in industrialised nations translate into a strong demand for drug development. The current industry expectation is that these factors, in combination with rapid technological advances in drug discovery, will dramatically increase the number of new compounds in an already robust pipeline. Over 280 products are currently in pivotal stage clinical trials in the US (May 2000). Each potential new product must undergo four stages of regulatory evaluation and each phase represents a potential project for Flex BioPharma.

Product

Flex BioPharma's success in the marketplace will be measured by its ability to enable clients to manufacture trial products more quickly than with current stainless steel technology. To that end, Flex BioPharma's product offering will initially include:

- Consulting Services – the project team will develop the disposables-based production process in close collaboration with the client's research laboratory. Once a validated process has been developed, it will be assembled at the client site under Flex BioPharma's supervision;
- In-situ Commissioning & Validation of the installed process to meet regulations set by agencies such as the American FDA (Food & Drug Administration);
- Supply Chain Management – Flex BioPharma's economies of time and scale will aggregate disposable equipment suppliers thus relieving the biopharmaceutical client from the burden of dealing with multiple manufacturers.

Human and capital resources requirements will, to a large extent, be dictated by the two-stage growth strategy pursued. Flex BioPharma's core business will initially centre on the consulting practice, until the economic benefits of disposable processes have been proven. It is estimated that each consulting project will last around 6 months and utilise 6 engineers/technicians.

Business Model

It is acknowledged that the consulting practice is only scalable to a point. Furthermore, if our growth projections for the market are correct, Flex BioPharma risks being squeezed out of an increasingly competitive market if barriers to entry are not created. Thus, we will continuously refine our products as part of solving client problems to ensure that Flex BioPharma's technology leadership is maintained. Once the company has achieved a level of credibility we will seek to become a dominant player in this market by investing in the acquisition of an R&D facility. This will help to guarantee our pipeline of new patents for the manufacturing of biopharmaceutical drugs using disposable equipment and create a valuable licensing business.

Management Team

Flex BioPharma brings to commercial fruition the ideas and expertise of its six founders.

The intellectual property of the company lies with its CEO, Joana Novais, a chemical engineer soon to earn her PhD in Biochemical Engineering from University College London. Her research work has focused on the engineering and economic aspects of the use of disposable equipment for the production of biopharmaceuticals. This research work has enabled her to establish extensive contacts with numerous disposable equipment manufacturers in Europe and the US.

Rob Schult, a naval engineer, has ten years experience in project and production management, specialising in analysis of controlled substances and assurance of contamination prevention. In addition to his MBA, he has two years experience as a

management consultant. His primary focus is process management, on site customer management, and project cost controls.

Gavin Watson spent the 10 years before his MBA working in the UK, US and Netherlands for environmental consulting firms specialising in wastewater treatment and bioremediation. He brings extensive project management, business development and client relationship management experience to the Flex BioPharma team.

Sergio Sperat, an information systems engineer and holder of an MBA, was intricately involved in two Latin American dot.com start-ups as Chief Technology Officer. In addition, he brings over 15 years of project management experience.

Francis McCullough, a structural engineer with an MBA, specialises in project valuation and corporate finance. He brings over 5 years of experience as a project manager in a multi-disciplinary engineering consultancy, designing and building chemical plants.

Scott McKinnon was Product Manager for a Fortune 500 company in the US managing blue-chip product lines which generated over $25 million in yearly revenue before taking his MBA. He designed the premier end-to-end 'in house' licensing division that resulted in $10 million in incremental revenue, and created the most successful Web-based promotion in Microsoft OEM history.

Financial Projections

Flex BioPharma represents a solid investment opportunity. A valuation based on earnings estimates the company's economic value to be $64.7 million by the end of Year 5. The first year of profitable operations will be two years after launch.

	Year 1	**Year 2**	**Year 3**	**Year 4**	**Year 5**
Total Revenue	**$614,400**	**$5,208,000**	**$16,023600**	**$28,018,800**	**$44,209,000**
Revenue from Consultancy	65.1%	69.1%	67.7%	67.3%	66.2%
Revenue from Supply Chain Management	34.9%	30.9%	28.8%	28.3%	28.0%
Revenue from Patents	0%	0%	3.5%	4.4%	5.8%
Profit	**-$266,000**	**-$397,350**	**$2,641,844**	**$7,336,656**	**$13,686,599**
Profit Margin	-43.3%	-8.0%	16.0%	26.0%	33.0%

At the outset, the principal driver for revenues is our consultancy arm. However, as the disposable process becomes more widely accepted, revenues from patent licensing will represent a greater proportion of the firm's income. Patent revenues are forecast to begin in Year 3, and additional patents and therefore revenues are expected to be generated through the R&D facility. The consulting arm is only scalable to a point and is very dependent on human capital. Therefore the licensing of disposable technology patents in this new field enables the business to scale up in ways not possible with the consulting arm alone.

Investment Opportunity

Once the process has been proven, investors will be offered the opportunity to invest $1.5 million to enable Flex BioPharma to establish itself as the primary channel for disposable biopharmaceutical drug manufacturing. Having already opened an office in London, this funding will be used to establish a presence in the key locations of the large US market, Northern California and on the East Coast. We anticipate that in the third year of operations, second round financing of $8 million will be required to establish an R&D facility to further patent research.

Risks

- Inability to meet claimed time to market targets – to address this our business model allows for 18 months to prove our technology and attain credibility before large scale investment in our own R&D facility;
- Substitute technology – substitutes for our patented products could be released before Flex BioPharma is able to gain market share. This is considered low risk since we have a three year technology advantage resulting in a strong product pipeline and our strategy incorporates continuous investment in R&D;
- Competitors reaction – contract manufacturers may react with price reduction. We believe the additional revenue opportunities achieved by disposable time savings will more than offset any price reductions achievable by contract manufacturers;
- Failure to adopt – biopharmaceutical firms could ignore the time to market benefits, or be averse to the new technology risk of disposable processes. This is perceived to be unlikely since both raising capital and finding available contractors is difficult and will become harder with increasing numbers of drugs;
- Staffing levels – the high demand for specialised bioprocess engineers may be a problem in the US but should not constitute a problem in Europe.

Exit

The average rate of return for both first and second round equity investors in Flex BioPharma will be approximately 50%. We consider there are three exit options:

- **Trade Sale A** – as disposable technology becomes more mainstream it is likely that a contract manufacturer will be interested in gaining expertise in the disposables without investing in the R&D learning curve.
- **Trade Sale B** –a large biopharmaceutical company or contract manufacturer will likely be interested in purchasing the combination of our disposable patent pipeline and proven consulting business as an investment.
- **IPO** – continuation of the business by the founders will required increased capital for further geographic expansion. The estimated value of the firm will be $64 million.

NOMENCLATURE

a	exponential decay constant for T_{obs}
a'	exponential decay constant for T_{real}
A	membrane area, in m^2
c	contingency factor (conventional plant)
c'	contingency factor (disposable plant)
C_b	solute concentration in the bulk, in gL^{-1}
C_b	solute concentration in the diafiltration buffer, in gL^{-1}
C_f	solute concentration in the feed, in gL^{-1}
C_p	solute concentration in the permeate, in gL^{-1}
C_r	solute concentration in the retentate, in gL^{-1}
C_{r0}	initial solute concentration in the retentate (at t=0), in gL^{-1}
C_r^A	solute concentration in the retentate available for transmission, in gL^{-1}
C_{r0}^A	initial solute concentration in the retentate available for transmission, in gL^{-1}
C_r^N	solute concentration in the retentate not available for transmission, in gL^{-1}
C_{r0}^N	initial solute concentration in the retentate not available for transmission, in gL^{-1}
C_w	solute concentration at the membrane surface, in gL^{-1}
C	solute concentration, in gL^{-1}
CF_n	net cash flow in year n, in £
$\mathcal{D}$	solute diffusion coefficient, in m^2s^{-1}
DC	direct costs, in £
DPC	direct production costs, in £
E_{conv}	equipment costs (conventional plant), in £
f_i	conventional plant factors for calculation of individual FCI items
f_i'	factors that translate conventional plant FCI items into disposable ones
FB	fringe benefits, in £
FC	fixed costs, in £
FC_{conv}	fixed costs (conventional plant), in £
FC_{disp}	fixed costs (disposable plant), in £
FCI	fixed capital investment, in £

FCI_{conv}	fixed capital investment (conventional plant), in £
FCI_{disp}	fixed capital investment (disposable plant), in £
FCI_n	fixed capital investment in year n, in £
g_i	factors for calculation of individual labour costs
g_i'	conversion factors for calculation of disposables individual labour costs
GOE	general operating expenses, in £
h_i	factors for calculation of FCI dependent costs
h_i'	conversion factors for calculation of disposables FCI dependent costs
IC	indirect costs, in £
J	flux, in $Lm^{-2}hr^{-1}$
k	mass transfer coefficient, in ms^{-1}
k_i	factors for calculation of ML dependent costs
L_{conv}	"Lang" factor (conventional plant)
L_{disp}	"Lang" factor (disposable plant)
m	year of entry to market
ML	maintenance labour costs, in £
MM	maintenance materials costs, in £
n	project year or process step
N	number of processing steps
N_D	number of diafiltration volumes
NPV	net present value, in £
OL	operating labour cost, in £
OL_{conv}	operating labour costs (conventional plant), in £
OL_{disp}	operating labour costs (disposable plant), in £
P_i	inlet pressure, in bar
P_o	outlet pressure, in bar
P_p	permeate pressure, in bar
Q	crossflow rate, in $Lmin^{-1}$
r	discount rate
R	retention factor
R_c	cake (concentration polarisation and fouling) resistance, in m^{-1}
R_m	membrane resistance, in m^{-1}
RC	running costs, in £
RC_{conv}	running costs (conventional plant), in £

RC_{disp}	running costs (disposable plant), in £
RC_n	running costs in year n, in £
S_n	value of sales in year n, in £
t	time, in minutes
t_P	processing step duration, in minutes
t_P	processing step n duration, in minutes
t_R	regeneration time, in minutes
T	transmission
$T^{(n)}$	transmission in stage n
T_0	observed transmission at t=0
T_0'	real transmission at t=0
$T_0^{(n)}$	initial transmission in stage n
T_{actual}	actual transmission (stagnant film model)
TMP	transmembrane pressure drop, in bar
T_{obs}	observed transmission
T_{real}	real transmission (model 2)
V	volume, in L
V_D	volume of diafiltration buffer, in L
VC	variable costs, in £
W	transmission recovery
x_i	fractions of conventional running costs
x	initial fraction of non-available solute
y	coordinate in the direction perpendicular to the membrane surface
y_i	conversion factors for calculation of disposables running costs fractions
Y	process yield

Greek letters

δ	thickness of the boundary layer, in m
η	dynamic viscosity, in Pa.s
τ	project life, in years

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