The University of New South Wales

Graduate School of Biomedical Engineering

Development of a Prototype Hollow Fibre Bioreactor System

Thesis submitted as partial fulfilment of the requirements for the Degree of Master of Biomedical Engineering by Course Work at the University of New South Wales, Sydney.

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Certification

I hereby declare that this submission is my own work and to the best of my knowledge it contains no material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma at the UNSW or any other educational institution, except where due acknowledgment is made in the thesis. Any contribution made to the research by others, with whom I have worked at UNSW or elsewhere, is explicitly acknowledged in the thesis.

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Abstract

Mammalian cell separation and high-density expansion systems that conform to stringent pharmacological standards of Good Manufacturing Practice (GMP) are required in modern disease treatments where cultured cells are used in addition to pharmacological agents. This document presents the design and development of a hollow fibre bioreactor as a solution for a high-density cell expansion system, in which a sterile homeostatic intra-capillary environment is maintained.

Physical, metabolic and biological characteristics of the cell line dictate the technical and engineering requirements of the bioreactor. Mathematical and computational models of the system are used to simulate and evaluate the system design. The materials in direct contact with cells or media have been selected to ensure biocompatibility and sterility.

In the intra-capillary hollow fibre bioreactor system, cells are located in the lumen of a hollow fibre module; a module similar to that used for haemodialysis. Metabolites and gases are transported in and out of the hollow fibre module by perfusion, and diffuse across the semipermeable membrane of fibres into the intra-capillary space where cells reside. Performance of the system is optimised by the appropriate control of the intra-capillary and extra-capillary perfusion rates and concentration of media components. The temperature is also controlled as part of the homeostatic intra-capillary environment conditions.

Cells were expanded in the system prototype with no intra-capillary perfusion. Cell growth was evaluated by measurement of glucose consumption and lactate production from the extra-capillary circuit.

The intra-capillary hollow fibre bioreactor is still at a very early stage of development. Modelling tools and temperature control systems provide a sound theoretical and technical basis for future development of a system that meets the stringent requirements for cell expansion.

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1 Introduction

1.1 Need For A Cell Expansion System

Cell culture is a well-established practice (1) used in many different biotechnology fields. In recent years, advances in molecular cell biology have led to development of new treatment strategies that use cultured cells in addition to pharmaceutical agents. Just as pharmaceutical agents require stringent standards for their production, therapeutic cell subsets will need development of processes that conform to pharmaceutical standards of Good Manufacturing Practice (GMP). Therefore cellular manipulations should be conducted in closed-sterile systems that are automated to minimise operator error. In addition to these GMP requirements, cell culture systems should be optimised so that cellular therapies will be economic.

Development of a cell expansion system for clinical therapy would provide a core technology for cancer research and treatment (2) (3), bone marrow transplant (4) (5) (6) (7), gene therapy (7) (8) and the treatment of HIV infection (8).

1.2 The Intra-Capillary Hollow Fibre Bioreactor

The intra-capillary hollow fibre bioreactor consists of a hollow fibre module (section 7.1) in which a homeostatic environment is maintained within the intra-capillary space of hollow fibres via intra-capillary and extra-capillary media perfusion. Metabolites, metabolic waste and gases are transported in and out of the intra-capillary space of the module by diffusion to and from perfused media. In addition to control of metabolite exchange, temperature and growth factor concentrations are other critical control parameters.

1.2.1 Why An Intra-Capillary Hollow Fibre Bioreactor System?

A variety of culture devices are available for cell expansion, but as yet, these technologies are not economic and do not always meet the stringent requirements imposed by GMP. Table 1 shows different culture devices for *ex vivo* cell expansion and their main related problems.

Growth of cells within the intra-capillary space of a hollow fibre module may overcome many of the problems associated with standard tissue culture techniques. Stan-

dard tissue culture techniques are bulky and expensive, and a break in sterility occurs every time media is replenished.

Culture device	Cell density (cells/ml)	Main Problem	Ref.
Tissue culture flask	$<10^6$	Expensive to scale-up.	(9)
Gas permeable bags	$<10^7$	Expensive to scale-up. Not suitable for adherent cell lines	(2)
Spinner flask	$<10^6$	Expensive to scale-up. Not suitable for adherent cell lines	(10)
Parallel plate systems	10^7	Requires established stromal feeder layer for support of haematopoietic cells. Not suitable for cytokine driven culture systems used for gene transfer.	(11)
Hollow fibre (extra-capillary growth)	10^8-10^9	Not possible to harvest adherent cell types. Regions of inadequate media perfusion with cell necrosis.	(3), (12), (13)

Table 1 – Culture devices for *ex vivo* manipulation of human cells

Intra-capillary cell growth offers the possibility of using the same expansion module for pre-enrichment of target cell types (14) and has the following potential advantages.

Mass transfer of culture components: A system based on a semipermeable membrane allows optimisation of the use of expensive materials by independent control of metabolite and growth factor exchange. The specific molar uptake (or production) as well as the concentration of various culture components varies over orders of magnitude, making independent control of mass transfer of specific culture components highly desirable. For example, a membrane that excludes molecules with a molecular weight greater than 10,000 could be used to prevent loss of more expensive components (growth factors and albumin) by confining them to the intra-capillary space where cells are grown, whilst low molecular weight substrates (oxygen, glucose, lactate) that can diffuse across the membrane are perfused on the outside of fibres. In this way expensive culture components are not discarded once metabolites have been depleted.

High density culture: Hollow fibre modules have a high surface area to volume ratio for exchange of nutrients and gases, and therefore can support growth of cells at a

density that is 10- to 100-fold greater than static culture systems. Hollow fibre bioreactors can be used for individualised patient care and would significantly reduce the laboratory space and number of incubators required for *ex vivo* manipulations.

Storage of media: Because hollow fibre bioreactor mass transport is based on media perfusion from reservoirs to the hollow fibre module, media can be stored at lower temperature (4ºC) than the bioreactor (37ºC) so that degradation of media components is minimised.

Manufacture of cell expansion modules: A further advantage of the hollow fibre bioreactor is that the expansion module can be mass produced and some hollow fibre materials (eg., cellulose) have been approved for used in extra-corporeal therapies (eg., dialysis).

1.2.2 Building An Intra-Capillary Hollow Fibre Bioreactor

A step by step description of the procedure is presented in Table 2.

Step No.	Objective
1	Cell line characterisation (physical, metabolic, biological)
2	Expansion goal (seeding densities, and expected final biomass and cell densities)
3	Mass transfer requirements for O_2, CO_2, glucose, lactate, and other small (MW<10.000) and large (MW> 10.000) molecules.
4	Design and define operating parameters for the hollow fibre bioreactor perfusion system; media composition, flow rate, temperature, cell inoculation concentration etc.
5	Design and define operating parameters for gas and temperature exchange module.
6	Monitoring of media composition and biomass.
7	Refinement of operating parameters

Table 2 – Steps in bioreactor development

The first step in the design and development of the hollow fibre bioreactor is to characterise the cell line with parameters such as cell size, sedimentation rate, specific glucose and oxygen uptakes and others, that are relevant for this process.

Next, the expansion goal, ie the expected final cell density and biomass for a specific seeding density, is defined.

The specific uptake (or production) of each component will define the mass transfer requirements to maintain homeostasis so that nutrients are not significantly depleted

and toxic metabolites are not accumulated. As cells grow at an exponential rate, mass transfer requirements will change over orders of magnitude during the culture period, which will determine the operation parameters of the system.

These parameters provide a basis for design and selection of mass transfer elements, definition of media composition, flow rate of media and other technical issues.

Gas and temperature media conditioning requires specific gas and heat exchange elements upstream to the hollow fibre module. For a functional analysis of the system, a sample-collecting unit to allow sterile sampling of the extra-capillary media is required.

The testing process of the design consists of the comparison of the system against its mathematical and computational model. Any discrepancy between the two implies a remodelling or adjustment of the system, until the final functional requirements are met.

1.3 Aims Of The Project

The general aim of this project is to develop a prototype hollow fibre affinity bioreactor system for growth of mammalian cells. Specific aims are as follows.

I. Characterisation of cell line properties that are relevant for hollow fibre bioreactor design.
II. Development of a mathematical and computational model of mass transfer for cylindrical geometry to be used as a design tool.
III. Development of a temperature close loop control for the hollow fibre bioreactor.
IV. Design and development of the perfusion system (hardware and software).

1.4 Document Structure

This document presents the design and development of a prototype hollow fibre bioreactor system. As well as guiding the reader through the different stages of development, a set of tools is provided to facilitate future refinement of bioreactor components.

Background for the design and development of the system and a basic description of cell culture techniques are presented in chapter 2. The concept of the intra-capillary hollow fibre bioreactor is introduced in chapter 3 with an overview of the system and its parts. The cell line characterisation, which is the biological base for the system

design, is presented in chapter 4. Chapter 5 analyses mass transfer in a cylindrical geometry as the mathematical model for mass and heat transfer in hollow fibres and silastic tube. The closed loop temperature control system is designed in chapter 6 as an example of the control of a homeostatic variable. Chapter 7 describes the prototype intra-capillary hollow fibre bioreactor where the concepts presented in previous chapters are applied, and a cell expansion experiment is presented in chapter 8 to illustrate the system's use.

The design, development and final prototype is evaluated in chapter 9 and the conclusions of the project are presented in chapter 10.

2 Background

This chapter presents the necessary background for the design and development of an intra-capillary hollow fibre bioreactor system.

2.1 Supporting Theory

The design and development of the hollow fibre bioreactor system requires a multi-disciplinary approach. This project has drawn on control system theory, mass transfer theory, cellular biochemistry and programming tools for the system development.

2.1.1 Control System Theory

Control theory was used in the development of the mathematical model and algorithm for the temperature control unit. This includes system identification (15) in the *z* domain (16) as the discrete form of the Laplace transform (17) and the control design using root locus theory (16). This same theory can be used if other parameters have to be controlled. Because of the non-linear characteristic of all the parameters in the system, a more complex non-linear control theory and model (18) should be used to improve and optimise the control of the expansion system.

Discrete modelling and control was used because the system was controlled with a digital computer, which is inherently a discrete machine (16) (19) (20). The detailed design and development of the temperature control system is explained in section 4.5 of this document.

2.1.2 Mass Transfer Theory

The perfusion of media and diffusion of molecules in media and across the hollow fibre membrane are the basis of nutrient, waste and gas transport to the expanding cells. Concepts of diffusion of a substance in a liquid and permeability (21) are used in the mathematical model of diffusion in a cylindrical geometry (22), where fluid flow is considered to be laminar (21). The concepts of gas solubility and partial pressures (21) (22) are used for gas transport.

2.1.3 Cellular Characterisation

The metabolic and growth factor requirements of the cell expansion process are required for design of the final system. The cellular uptake or production of a substance depends on multiple environmental factors including temperature and the metabolic and growth factor milieu of the cellular microenvironment. Furthermore, cells may differentiate in culture changing their physical and biochemical characteristics. Accurate physical and metabolic cellular characterisation is required to create an appropriate cell line model to be used in the system design (section 5.1).

In vitro and *in vivo* cell characteristics are not identical (23) because the cell microenvironment *in vivo* is not exactly replicated by *in vitro* experiments.

The heterogeneity of cell populations means that any measured cellular parameter will vary from cell to cell. Flow cytometry provides a technology for measurement of the distributions of cell number, size, granularity and surface molecule expression. Metabolic characteristics such specific O_2 uptake, CO_2 production, glucose uptake, lactate production are more easily measured in bulk culture, though do not reflect individual cellular characteristics but average values for cell populations.

The microenvironment in which cells proliferate can be considered as part of the cell characterisation. Partial pressures of O_2 and CO_2, pH, temperature and anchoring surfaces are examples of microenvironment factors that influence cell growth.

Those cellular properties that are not considered in the initial design such as growth factor consumption may require adjustment of hollow fibre system operating parameters. Although only the interaction between the cell and its environment is of importance for the cell expansion system, a more complete description of cellular physiology is only possible by understanding cellular biochemical pathways.

2.1.4 Programming Tools

The hollow fibre bioreactor system is a cell-expansion machine. As a machine it has internal control logic as well as a user interface. A PC computer running LabVIEW® was chosen as the platform for the control unit and user interface of this machine. Analogue and digital data acquisition (24) are used for the interaction between the PC computer and the cell environment controllers.

MATLAB® was the tool used for the computational modelling and analysis of the system.

These programming tools were the option used for this specific project, but other software tools can also be used for future developments.

2.2 Techniques

There are many useful techniques available for cell characterization and culture (25). It is beyond the scope of this document to provide a full description of each technique, though the cited references do provide more detailed descriptions.

2.2.1 Cell Counting Techniques

Cell counting techniques are of vital importance in the evaluation of any cell expansion system. Table 3 shows some of the techniques used.

Name	Description	Advantages	Disadvantages	Ref.
Grid Cytometer.	Counting under the microscope the occurrences in a known volume defined by a marked glass grid.	- Easy to use	- Inaccurate - Slow	(22)
Microscope image recognition.	Software/hardware recognition of cells in a predefine volume.	- Easy to use - Fast	- Reduced range - Inaccurate	Appendix E.1
Sytox-Green assay.	Measurement of fluorescence of stained cells.	- Accurate - Good range	- Slow - Requires pre-counting preparation	(26)
Flow Cytometer	Counting of cells in a volume defined by the counting of beads at a known concentration.	- Fast - Accurate - Little preparation	- Requires adding of beads for counting reference.	(27)

Table 3 – Cell counting techniques

2.2.2 Cell Handling Techniques

Whenever mammalian cells are transferred there are a number of potential hazards. The culture system can become contaminated or the culture itself may present a significant biological hazard (eg., HIV infectious risk).

An understanding of the microorganisms that can contaminate the cell culture provides a basis for implementation of adequate procedures for maintaining sterile cultures and infection control. Bacteria and fungus are the main two contaminants affecting experimental cell cultures systems, though it must be assumed that viruses such as hepatitis B (HB) and human immunodeficiency virus (HIV) can be present in cultures derived from human sources.

All culture components must be sterile and free of microorganisms. Bacteria and fungi are filtered out of media components using 0.3μm sterile filters, and all pipettes and culture containers are sterilised using either gamma irradiation or autoclaving. There are chemical sterilisation techniques such as hydrogen peroxide or ethylene oxide that may be appropriate for certain plastics that are damaged by harsher sterilisation methods.

The cell culture vessel should be hermetically "sealed", though this is not always possible when tissue culture flasks require transfer of CO_2 within a CO_2 incubator, though culture vessels can be vented using 0.3μm gas filters. Whenever the culture is transferred between tubes or flasks, the procedure is carried out within a laminar flow hood that filters out airborne bacteria or fungi. Furthermore, procedures should avoid touching any component of the culture system with non-sterile implements .In any case, the number of cell culture transfers should be minimise to reduce the chance of contamination.

2.3 Basics Of In Vitro Cell Growth

The KG1a cell line (28) was used throughout the development of the cell expansion system. This is an immortal cell line that is easily grown using standard tissue culture reagents (RPMI+10%FCS). Although some aspects of cell metabolism are well represented by this cell line, other cell types will require modification of system operating parameters.

2.3.1 General Principles

As a general rule, all cell manipulation has to be done in a sterile environment to prevent contamination, and a sampling technique that does not contaminate the culture is required for expansion assessment. The four main steps required for *ex vivo*

cell production are cell selection, cell inoculation of the culture device, cell expansion and harvesting. Cryopreservation is used for long-term cell preservation.

Selection of Cells

Different types of cells can be cultured at the same time, but often a target cell type needs to be selected for manipulation in culture. If the target cell type is rare, then cell selection will significantly reduce the volume and cost of the culture system. Important performance parameters of cell separation techniques are yield, purity and cell viability.

Inoculation of the culture device

Similar to *in vivo*, *in vitro* cell growth requires a structure that localises expanding cells to form a cellular microenvironment that provides the necessary growth factors and nutrients for growth. This can be called the *"expansion chamber".* The inoculation process consists of placing the cells in the "*expansion chamber*", which could be either a tissue culture flask, the lumen of a hollow fibre or any other space in which a homeostatic environment can be maintained.

Cell expansion

Cell expansion rate is critically dependent on the cellular microenvironment, and therefore its quality determines the efficiency of the expansion system. Some cell lines require an anchoring surface (anchorage dependent) or cellular stroma for growth. Depending on the cell line, the type of media and other factors, different media replenishment strategies are used to maintain this environment. For mammalian cell lines, temperature, pH, pCO_2 (depending on media buffering) and pO_2 have to be controlled.

Harvesting

Once cells have grown to confluence, or have significantly depleted media components they are harvested. For adherent cell lines, harvesting will require techniques for detaching cells from their anchoring surface.

Cryopreservation

A full description of cryopreservation techniques is beyond the scope of this thesis. In brief, cells are suspended in a high concentration of FCS and 10% DMSO to prevent ice crystal formation during the freezing process. Cells can then be stored in liquid nitrogen for many years.

2.3.2 Tissue Culture Flask Culture Of KG1a

The growth of KG1a cells in tissue culture flasks is a well-established practice (22) in which the four main steps of cell culture are followed. This cell line grows in suspension (expansion) and does not require techniques for removal of cells from the tissue culture flask surface. The cell line is maintained in culture by harvesting cells before the culture exceeds 10^6 cells/ml and then cells are reinoculated (selection and inoculation) at a density of 10^4-10^5 cells/ml.

2.3.3 Growth Of Cells Within The Intra-Capillary Space Of Hollow Fibre Modules

The four main steps (cell selection, inoculation, cell expansion and harvesting) can be combined using a hollow fibre affinity bioreactor. Because of the novelty of this process it is worthwhile specifying this procedure.

Selection

The hollow fibre affinity cell selection process is described in detail elsewhere (14). The device consists of a number of hollow fibre membranes running in parallel through a cylindrical housing. A molecular ligand (eg. monoclonal antibody) is attached to the inside of fibres, and is capable of capturing specific cell types as they pass along hollow fibres.

A medium-scale device (surface area 400cm^2) has been tested for bone marrow stem cell enrichment from mobilised peripheral blood using monoclonal antibody to CD34. The CD34$^+$ cell enrichment purity was at least 90% (1000 fold enrichment) with a yield of 60%.

Inoculation

This is a very delicate process because cells are to be uniformly deposited in the lumen of hollow fibres where cell growth will take place. The efficiency of this process

is directly reflected in the final system performance. Accurate cell deposition requires a sequence of precisely controlled fluid displacements achieved using computer-controlled pumps and valves.

Cell expansion

The expansion of the cells is strongly dependent on the quality of the system that controls homeostatic variables such as temperature, pO_2, pCO_2, nutrient concentrations, waste removal and flow rates of intra and extra-capillary fluids (media). Set points, which are defined by the physiological requirements of cells, are specified for the control of those homeostatic variables. A computer controlled, closed-loop system is used to maintain the variables within an accepted range around the specified set point.

Harvesting procedure

The harvesting of the cells is done simply by a rapid saline wash of the intra-capillary space of the hollow fibre module. Depending on the final use of the harvested cells, this process may have to be automated and under sterile conditions.

Sampling

Although not part of the expansion itself, sampling of culture media is important to control and optimise the culture process. Samples were taken from the extra-capillary media to evaluate glucose and lactate concentration. It is critical that sampling is done using a sterile process.

The following chapter presents an overview of the hollow fibre bioreactor system, as an introduction to the coming chapters, in which the background concepts presented here are applied.

3 Intra-Capillary Hollow Fibre Bioreactor Overview

An overview of an intra-capillary hollow fibre bioreactor system is presented in this chapter. This will give the reader a global view of the system and a better understanding of the importance of the coming chapters.

3.1 Global View

A general diagram of the intra-capillary hollow fibre bioreactor system is shown in Figure 1.

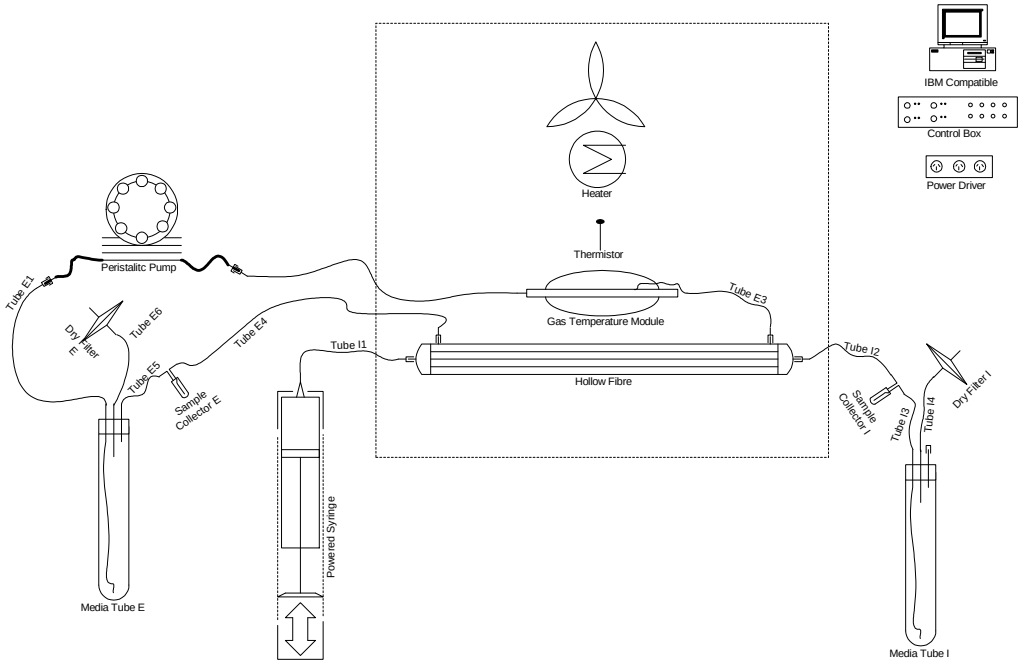


Figure 1 - Intra-capillary hollow fibre bioreactor diagram

From a functional point of view, the system consists of three distinct sub-systems which are i) the intra-capillary circuit, ii) the extra-capillary circuit, and iii) the control unit. Each of the sub-system is described in the coming sections, and a physical and functional description of the parts is given in chapter 7, when the prototype system is presented.

3.2 The Intra-Capillary Circuit

Table 4 summarises the parts and their functions in the intra-capillary circuit.

Part	Function
Powered Syringe	Media driving force – Media reservoir
Hollow Fibre Module	Cell expansion chamber.
Sample Collector I	Collect intra-capillary media samples.
Media Tube I	Waste collection.

Table 4 - Intra-Capillary parts & functions

The intra-capillary space of the hollow fibre module is the chamber where cells are located. Therefore, the intra-capillary volume has to me maintained with homeostatic conditions to ensure cell expansion. This implies an appropriate concentration of nutrients, gases, growth factors and other molecules, as well as a controlled temperature and pH of the media.

The intra-capillary circuit provides the media that goes in direct contact with the cells. Any molecule required for the growth with molecular weight (MW) greater than the cut-off MW of the membrane of the hollow fibre is supplied via this circuit.

Other molecules with MW less than the cut-off MW of the membrane, such as gases or glucose, are provided via diffusion from the extra-capillary circuit.

The temperature of the intra-capillary volume is also controlled via the extra-capillary flow. Heat can be considered and analysed as another element that is transferred between the extra-capillary to the intra-capillary space.

An important consideration in the intra-capillary circuit is the maximum possible flow rate of media, because this can “wash-out” the cells from the fibres due to shear force.

3.3 The Extra-Capillary Circuit

Table 5 summarises the parts and their functions in the extra-capillary circuit.

Part	Function
Media Tube E	Media reservoir.
Peristaltic Pump	Media driving force.
Gas and Temp Exchange module	Gases and temperature exchange to condition media.
Hollow Fibre Module	Source of metabolic substrates for intra-capillary space.
Sample Collector E	Sampling of extra-capillary media.

Table 5 - Extra-Capillary parts & functions

The media in the extra-capillary circuit provides the gases and nutrients that the cells require for growth, as well as other culture components that have a MW less than the cut-off MW of the hollow fibre membrane.

The mass transfer of gases, nutrients and waste between the intra-capillary and extra-capillary media is done via diffusion across the hollow fibre membrane. Therefore, this media has to be conditioned with the appropriate concentrations of culture components before reaching the hollow fibre module. The optimal temperature, pH, O_2 and CO_2 levels are set by the Gas and Heat Exchange module which is positioned inline and upstream to the hollow fibre module.

3.4 The Control Unit

Table 6 summarises the parts and functions of the control unit.

Part	Function
Computer (IBM)	Logic Unit.
Thermistor	Sensor.
Control Box	Signal adjustment.
Power Driver	Power control by digital signal.
Fan	Air flow.
Heater	Heat source.

Table 6 - Control Unit parts & functions

The control unit is a sensing, processing and actuating unit that controls the temperature of the hollow fibre module. The control unit can also be used to control any oth-

er variables such as pCO_2, pO_2 or pH, if required. A control unit consists in three main parts:

1- Sensor: Converts the sensed signal to a form understandable by the controller (eg: temperature to a voltage).
2- Controller: Reads the sensed signal and estimate the control parameter (eg: Read the voltage, calculate the required action, and generate train-of-pulses to the actuator).
3- Actuator: Converts the controlled parameter to the control action (eg: For each pulse of control, turn on and off a 240V heater).

The following chapters will provide the necessary components for the quantitative analysis of the interactions between the three sub-systems described above. These components are cell line characteristics (Chapter 4), mass and heat transfer in the hollow fibre bioreactor (Chapter 5), and process control (Chapter 6).

4 Cell Line Characteristics

The characterisation of a living cell is complex and includes its interaction with the local microenvironment and neighbouring cells (23). For example autocrine factors secreted by the cell will create a dependence of cell growth rate on cell density. Cell attachment factors or growth factors also influence entry into cell cycle and cell survival (23).

Even though a complete description of a cell line is not feasible, it is sufficient to determine the metabolic and growth factor requirements for *in vitro* culture as they form the basis for the design and development of the hollow fibre bioreactor.

4.1 Importance Of Modelling

A good model is the simplest representation of a system from which relevant information about the system can be obtained. Therefore it is not necessary to include all characteristics of a cell line to create a good model of it. A new model has to be experimentally validated before its predictions can be assumed to be correct.

4.2 Importance In Bioreactor Design

The hollow fibre bioreactor has to provide a homogeneous environment where gases, pH, nutrients, vitamins and growth factors in the intra-capillary space are optimal for cell growth. Initially design considerations are simplified by development of a simple model that will predict intra-capillary substrate concentrations. A more complete model will allow optimisation of design and performance of the bioreactor.

4.2.1 Cell Characterisation And The Influence On Bioreactor Design

Table 7 shows a list of cell characteristics that can be considered for modelling the properties of a cell line and their relevance for the hollow fibre bioreactor design.

Cellular Properties		Variable	Relevance to Bioreactor Design
Homeostatic variables		Temperature	• Temperature control system • Heat transport to intra-capillary space. • Media aging
		pH	• Media type • Buffering system
		pO_2	• O_2 concentration control in media • O_2 diffusion to intra-capillary space.
		pCO_2	• CO_2 concentration control in media • CO_2 diffusion from intra-capillary space. • pH
		Glucose concentration	• Media perfusion • Media aging
		Lactate concentration	• Media perfusion • Media aging
		Media type	• pH, pCO_2 control • Media aging
Cellular uptake	MW < memb. cut-off	O_2 specific uptake:	• O_2 mass transfer (29)
		CO_2 production	• CO_2 mass transfer • pH
		Glucose specific uptake:	• Glucose mass transfer • Media aging • Evaluate biomass
		Lactate production:	• Lactate mass transfer • Evaluate biomass
		Other molecules	• mass transfer
	MW > memb. cut-off	Other molecules	• intra-capillary mass transfer • osmotic pressure difference
	MW unknown	Autocrine growth factors	• intra-capillary and extra-capillary mass transfer
Cell growth:		Doubling time	• Adjustment of other parameter to biomass
Physical:		Shape	• Max number of cells in bioreactor
		Dimension	• Max number of cells in bioreactor
		Occupied volume	• Max number of cells in bioreactor
		Sedimentation velocity	• Time to settle in the fibre • Centrifugation
Other:		Attachment to membrane surface	• Membrane preparation • Expansion rate change

Table 7 – Cell characteristics and bioreactor relevance

4.2.2 Validation Of Hollow Fibre Bioreactor Design

Development of a model that adequately describes the bioreactor system is a process of refinement. The initial mathematical model is evaluated experimentally and modified if system variables do not come close to the models' prediction.

4.3 A Specific Example: KG1a Cells

4.3.1 Why KG1a?

KG1a cells were used to test the hollow fibre bioreactor system because they

- Express the CD34 antigen which is present on haematopoietic bone marrow stem cells
- Have similar metabolic properties to haematopoietic cells
- Are "Immortalised" and do not change their properties from generation to generation
- Are easily grown in tissue culture

The properties of other cell lines will differ from KG1a and will require different bioreactor operating parameters, however initial data as determined for KG1a will provide a starting point for growth of other cell types.

4.3.2 KG1a Characteristics

The data obtained to characterise KG1a cells used in this project are as follows.

Homeostatic Characteristics

This set of characteristics was taken from cell cultures in flask.

Homeostatic	Value	Units	Reference
Temperature	37±0.5	ºC	(22)
Media type	RPMI-1640		(22)
Glucose concentration	~11.0	mmol/L	Media Aging experiment (Appendix B.1.2)
Lactate concentration	~1.7	mmol/L	
pH	7.4		(29)
pCO_2	0.3	mmHg	Atmospheric (29)
pO_2	159	mmHg	

Table 8 – Homeostatic parameters for KG1a cells

As an example of the importance in maintaining well-controlled homeostatic conditions, and the effect of changes in these variables on cell growth, the results of the media aging experiments (Appendix B.1) are presented below. This experiment evaluates the effect of media (RPMI) aging at 37ºC on cell (KG1a) growth (Table 9).

Media Age (days)	Glucose (mmol/L)	Lactate (mmol/L)	Final Cell Density (cells/ml)
0	11.6	1.73	1.40E+06
3	11.2	1.68	1.24E+06
6	11.3	1.71	1.11E+06
9	11.3	1.73	1.20E+06
12	9.53	1.52	1.07E+06
15	5.76	0.88	9.90E+05

Table 9 – Media aging effects

Cellular uptake / production

Some metabolic parameters for KG1a cells are shown in Table 10. Many other metabolic parameters, such as production of autocrine growth factors are not known for this specific cell line.

Uptake / production parameter	Value	Units	Reference
O_2 specific uptake:	4.0E-17	mole/cell/hour	(22)
Glucose specific uptake:	1.0E-10	mmole/cell/hour	Appex B.1.1
Lactate production:	7.0E-11	mmole/cell/hour	Appeix B.1.1
Autocrine growing factors	Not known		
MW>10.000 (fibre cut-off dependence)	Not known		

Table 10 – Metabolic parameter for KG1a cells

It is important to bear in mind that the cellular uptake / production is not constant. The values presented on Table 10 are average values for specific conditions. The "Cellular uptake / production" experiment (Appendix B.1) evaluates the specific glucose uptake and lactate production in KG1a cells for different cell densities. Figures 2 and 3 show this dependence.

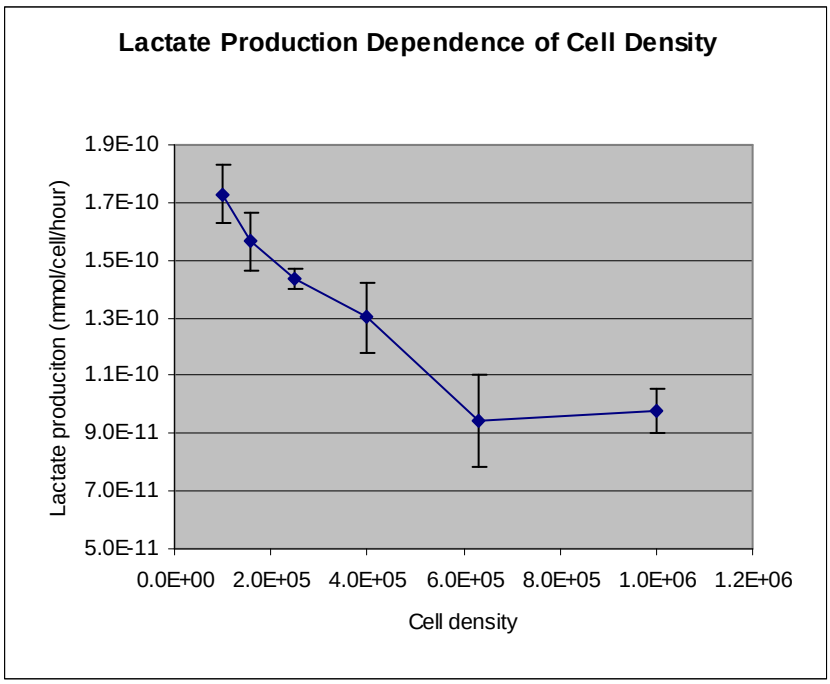


Figure 2 – Lactate production dependence of cell density

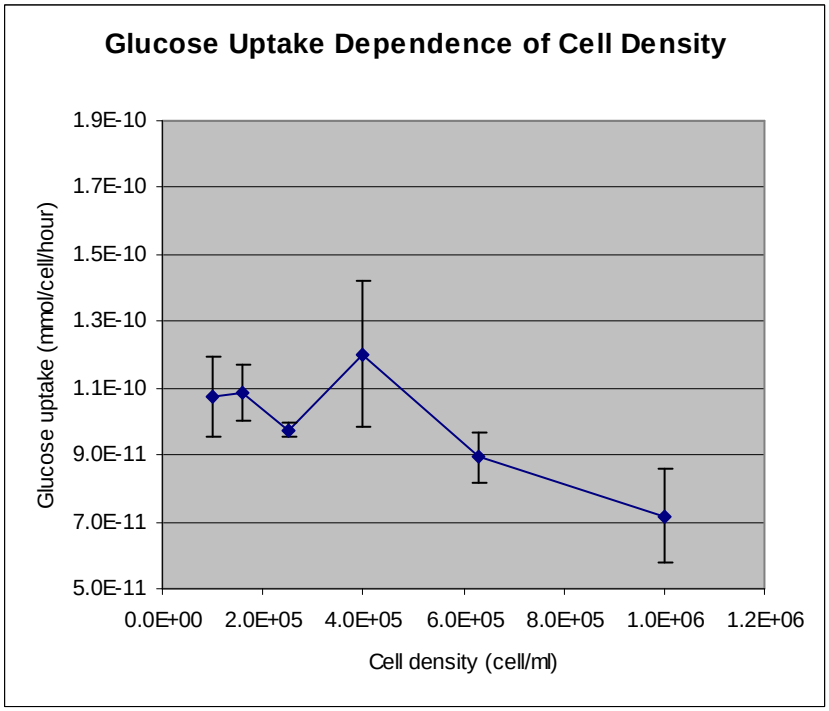


Figure 3 – Glucose uptake dependence of cell density

Growth Characteristics

Table 11 shows data obtained from cell in flasks.

Growth	Value	Units	Reference
Doubling time:	30	hours	(22)
Seeding density dependency	Refer to Seeding Density Dependence experiment (Appendix B.1)		

Table 11 – Growth characteristics for KG1a cells

As an example of cell growth variability, its dependence on cell seeding density was studied. Figure 4 shows the fold-expansion (output/input cell number) of KG1a cells related to seeding density.

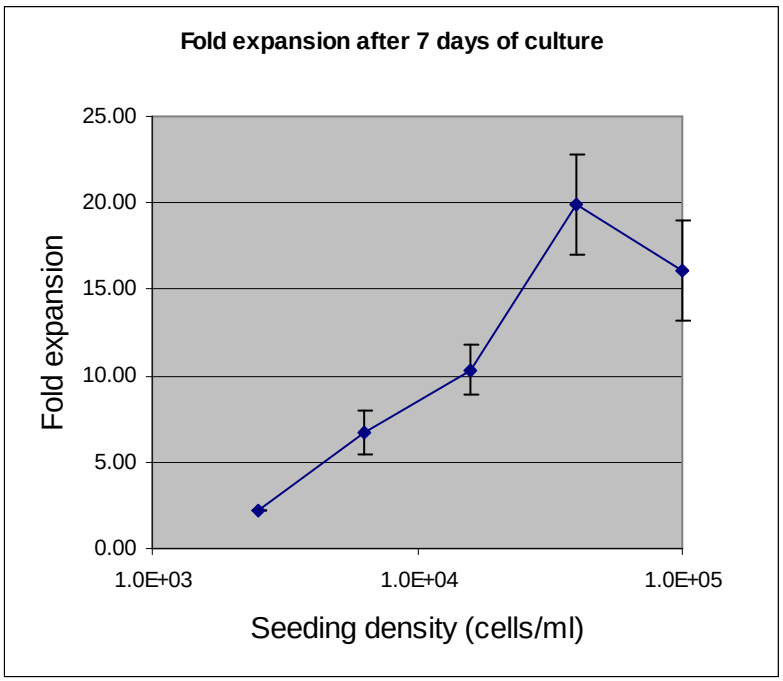


Figure 4 – Seeding Density Dependence

Physical Characteristics

Table 12 shows the physical characteristics of KG1a cells.

Characteristic	Value	Units	Reference
Shape:	Sphere (in suspension)		Microscopic observations.
Diameter:	8-14	μm	Other blood cells (29)
Pack cell volume:	1.0E+06	Cells/mm^3	Calculated from diameter.

Table 12 – Physical characteristics of KG1a cells

Once cell characteristics are determined the technical issues required to provide cells with an appropriate microenvironment for survival and expansion can be addressed. The following chapter analyses mass transfer in the hollow fibre module, an important technical aspect of hollow fibre bioreactor design.

5 Mass/Heat Transfer In The Hollow Fibre Bioreactor

Nutrients, growth factors, gases and other molecules have to be transported to and from the cell expansion chamber, the intra-capillary space, for cell survival and growth. Heat transfer is also considered in this chapter because governing equations are identical to the mass transfer model. Figure 5 and Table 13 describe the main elements of the system that are required for mass and heat transfer.

No.	Mass transfer element	Function
1	Hollow fibre module	*Perfusion:* Molecules with a MW greater than the cut-off MW of the membrane are perfused via intra-capillary media (single pass). Extra-capillary media is recirculating so that axial concentration gradients are minimised. Gas, nutrients and waste products diffuse to and from the intra-capillary media across the ultrafiltration (dialysis) membrane. *Diffusion:* Only those molecules with a MW less than cut-off MW of membrane diffuse between extra-capillary and intra-capillary spaces.
2	Gas Heat Exchange Module	*Diffusion:* Gases to and from extra-capillary media. *Media Heating:* Extra-capillary media is heated up to 37ºC down stream the hollow fibre module.
3	Peristaltic Pump	*Perfusion:* Extra-capillary media driving force
4	Powered Syringe	*Perfusion:* Intra-capillary media driving force.

Table 13 – Mass transfer components description

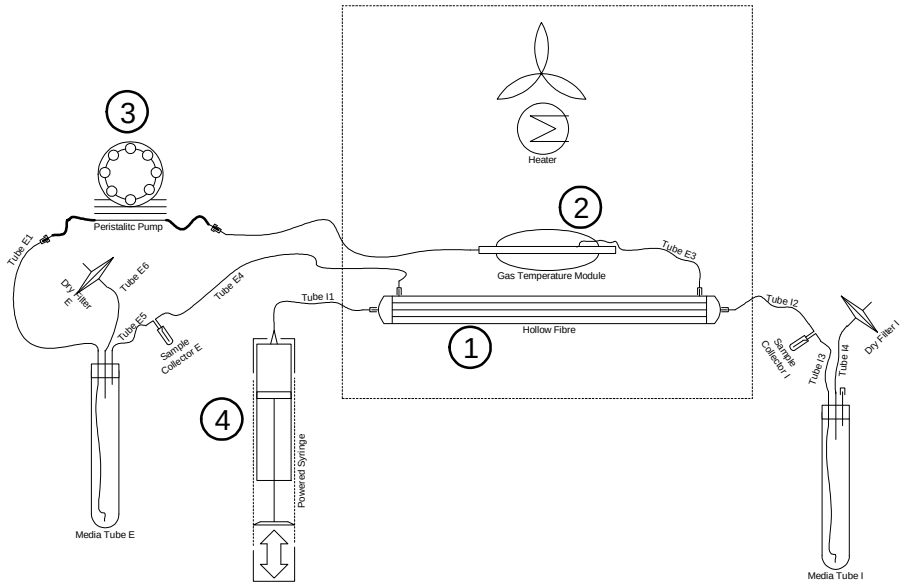


Figure 5 – Mass transfer in bioreactor

- Perfusion

Media is perfused from the reservoirs to the hollow fibre module. The specific cellular uptake of culture components (section 6.3) and the system mass transfer characteristics define the perfusion flow rates for both intra-capillary and extra-capillary spaces.

Pressure differences between inlet and outlet due to perfusion flow rate have to be evaluated to account for the effect on gas concentration and ultrafiltration rate.

Flow in the expansion system can be either turbulent or laminar. This is defined by the mechanical properties of the conduit and the flow rate (21).

Extra-capillary perfusion may create micro-currents in the intra-capillary space that can displace the cells and disturb the homogeneity of the cell distribution.

- Diffusion

Without convection in the immediate neighborhood of cells, all the molecules that are consumed (or produced) by the cells are transported by diffusion from the cells' surrounding microenvironment. Molecules in the extra-capillary circuit diffuse across the hollow fibre membrane. Gas transfer, for media conditioning, diffuse through the silicon tube in the GHE module.

- Ultrafiltration

Ultafiltration is not considered as an important mass transfer contributor in the hollow fibre bioreactor, because the difference in pressures between the intra-capillary

and extra-capillary spaces are small in comparison to pressure driving force required to create an ultrafiltration flux.

- Osmotic pressure

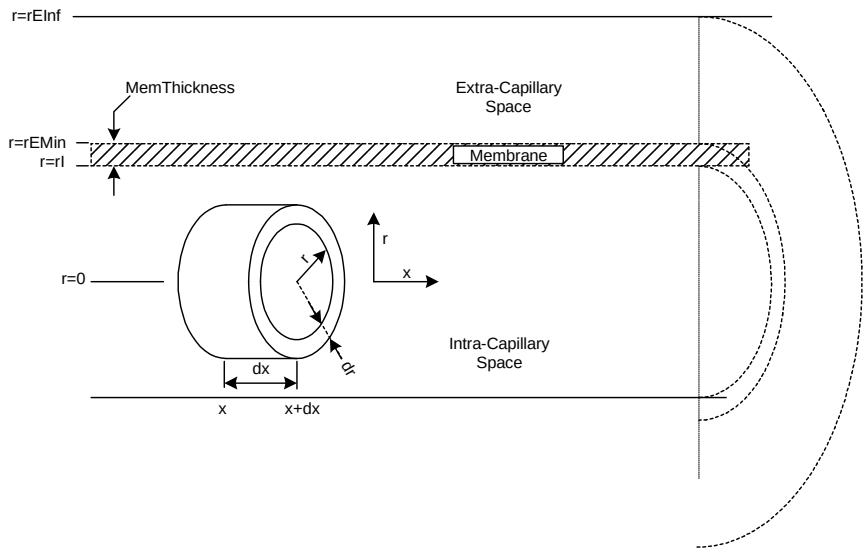
Osmotic pressure and its mass transfer effects are not considered in this document. Although osmotic pressure can be very important, it was not considered because the media used in both intra-capillary and extra-capillary circuits was identical. The osmotic pressure produced by cellular uptake/production of molecules that do not diffuse across the membrane was not studied.

5.1 Mass Transfer In A Cylindrical Geometry

The model of mass transfer in a cylindrical geometry is presented here because of its relevance for the study of the following elements of the bioreactor:

- Hollow fibre module.
- GHE module.

Figure 6 shows the studied geometry and an infinitesimal sub-cylinder at a position x with radius r. Radial diffusion, axial diffusion, and axial convection are considered in this axisymmetric, two-dimensional model.



x: longitudinal distance

r: radius from centre of cylinder

Figure 6 – Cylindrical Geometry

The terms for mass transfer in the infinitesimal cylinder are given in Eq.1 to Eq.4, where:

C: concentration
J: flux
v: fluid velocity
P_m: membrane permeability
J_m: membrane flux

- Axial convection:

  in at x: $2\pi r\, dr\, v(r) C(r,x)$
  out at x+dx: $2\pi r\, dr\, v(r) C(r,x+dx)$ **Eq. 1**

- Axial diffusion

  in at x: $2\pi r\, dr\, J(r,x)$
  out at x+dx: $2\pi r\, dr\, J(r,x+dx)$ **Eq. 2**

- Radial diffusion

  in at r: $2\pi r\, dx\, J(r,x)$
  out at r+dr: $2\pi (r+dr)\, dx\, J(r+dr,x)$ **Eq. 3**

- Accumulation: $2\pi r\, dr\, dx \frac{\partial C}{\partial t}$ **Eq. 4**

$$\frac{\partial C}{\partial t} = -v(r)\frac{\partial C}{\partial x} - \frac{1}{r}\frac{\partial (rJ)}{\partial r} - \frac{\partial J}{\partial x} \quad \textbf{Eq. 5}$$

For steady state:

$$v(r)\frac{\partial C}{\partial x} = -\frac{1}{r}\frac{\partial (rJ)}{\partial r} - \frac{\partial J}{\partial x} \quad \textbf{Eq. 6}$$

Fick's law:

$$J = -D\left(\frac{\partial C}{\partial r} + \frac{\partial C}{\partial x}\right) \qquad \textbf{Eq. 7}$$

Replacing Fick's law in Eq.15:

$$v(r)\frac{\partial C}{\partial x} = D\left\{\frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\left(\frac{\partial C}{\partial r} + \frac{\partial C}{\partial x}\right) + 2\frac{\partial^2 C}{\partial r \partial x} + \frac{\partial^2 C}{\partial x^2}\right\} \qquad \textbf{Eq. 8}$$

If intra-capillary and extra-capillary spaces are to be considered, Eq. 8 has to be solved for both regions and diffusion across membrane calculated.

- Membrane diffusion

Flux across memb. $J_m = P_m \Delta C$ **Eq. 9**

Eq. 8 has to be integrated to solve the concentration problem for which two different methods were used:

- Sherwood number solution

This solution only applies for intra-capillary space with a parabolic flow profile. It does not consider membrane characteristics or extra-capillary perfusion. Even though Eq. 8 has to be solved, that work is already done and can be immediately used (21). This approximation cannot be modified to account for material uptake or production, as it is the case when cells are considered as part of the system.

- Computational model.

Because of the importance and usefulness of the implemented computational model in the solution of the mass transfer problem, the next section (5.2) is dedicated to explain it in detail.

5.2 Computational Model

An axisymmetric computational model of concentration (or temperature) for a cylindrical geometry was developed to analyse the mass transfer and heat exchange for the hollow fibre module, the GHE module and the media-transport silicone tubing. This model is based directly on the specifications of the mass transfer terms (Eq.1 –

Eq. 4). A computational algorithm (Appendix F.3) simulates the concentration of a substance for both the intra-capillary and extra-capillary spaces. A non-parabolic flow profile and uptake/production of a substance can be simulated with this computational model. Figure 7 shows a simulation for O_2 concentration in a hollow fibre.

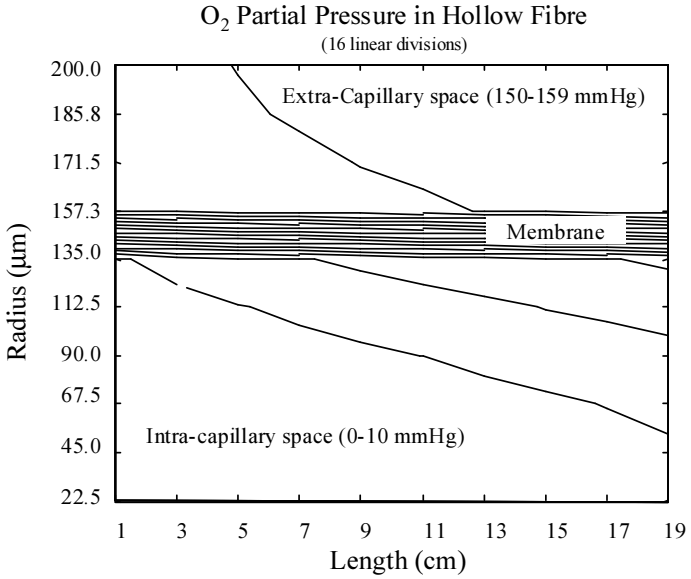


Figure 7 – O_2 in Hollow Fibre

The model was validated using O_2 mass transfer experiments (22). The final intra-capillary concentration of the model has an error of about 20% with respect to the experimental comparison data. Although this is a large error, the results are within an order of magnitude and represent a useful first approximation of system operating variables. Table 14 summarises the comparison between the modelled hollow fibre module and the experimental values obtained.

Experiment Description (Qb: intra-capillary flow rate, Qd: extra-capillary flow rate)	**Calculated value (Module Clearance O_2)**	**Experimental value (Module Clearance O_2)**
Module Nr.:95/0153, Qb=120 (ml/min), Qd=118 (ml/min)	12.5 (ml/min)	16.3 (ml/min)
Module Nr.:91/0628, Qb=120 (ml/min), Qd=118 (ml/min)	5.1 (ml/min)	6.3 (ml/min)

Table 14 – Hollow fibre model vs experimental

5.3 Mass Transfer In The Hollow Fibre Module

The hollow fibre module can be considered as a collection of fibres enclosed in a shell. The mass transfer characteristics of each fibre can be modelled as a cylindrical geometry element (see 5.1). Figure 8 shows how the total extra-capillary area is calculated. The extra-capillary area corresponding to each fibre is the total extra-capillary area divided by the total number of fibres of the module.

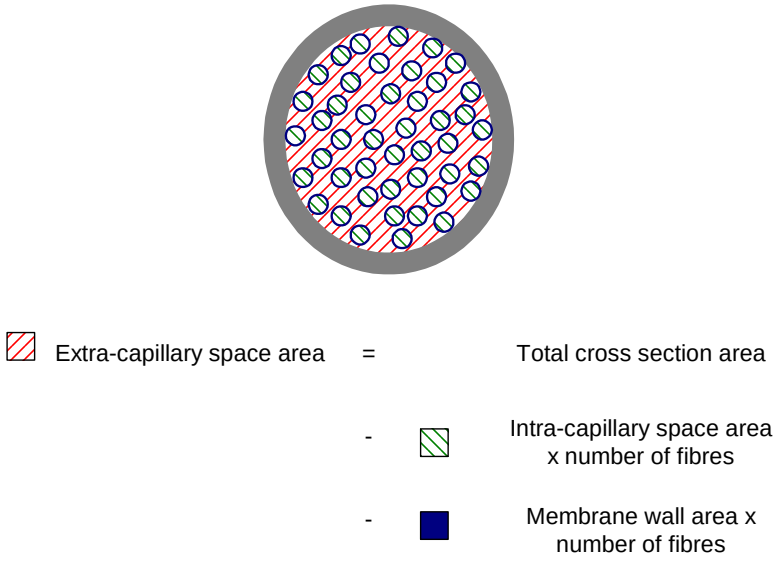


Figure 8 – Areas in cross-section of hollow fibre module

5.4 Supply Of Nutrients To Cells

The total mass transfer requirements for any molecule is determined by the biomass.

*Cell Specific Uptake of X * number of cells = mass transfer requirements of X*

where *X* is any substance consumed (or produced) by the cells.

Cells have to be in a homeostatic environment for optimal growth and cells will alter this environment by consumption (or production) of metabolites and growth factors. To compensate for these changes, mass transfer of the depleted or produced metabo-

lites and growth factors are necessary. Because autocrine factors are sometimes essential for cell growth, then it may be necessary to limit its clearance.

If mass transfer is inadequate then there will be

- not enough nutrients, gases and other molecules for cell survival.
- an accumulation of metabolic waste in media which may inhibit growth or be cytotoxic.

If mass transfer is too high then

- autocrine factors will be lost.
- cells may detach and flow out of the module by fluid shear stress.

The use of expensive culture components can be reduced adjusting the extra-capillary and intra-capillary flow rates and their concentration in media. Furthermore it will not be necessary to include albumin and growth factors in the extra-capillary circuit since these components will not cross the dialysis membrane. The osmotic difference could be made up by addition of dextran to the extra-capillary media.

The hollow fibre mass and heat transfer characteristics and cell line requirements define the homeostatic variables for cells to growth. The next chapter presents the design and development of the temperature control system as an example of the control of a homeostatic variable.

6 Process Control

The time constant for each of the processes involved in cell expansion varies over several orders of magnitude. Therefore many of the processes require independent control to maintain homeostatic conditions for cell growth. Slow processes, such as depletion of culture components, can be controlled manually. Fast processes, such as temperature variation, require a computerised close loop control to maintain the controlled variable within the appropriate range.

The complete temperature control system described below is an example of how a controller is designed and implemented.

6.1 Set Point And Range Definition

Temperature is an important homeostatic parameter for cell expansion. For mammalian cells the temperature have to be maintained at 37 ± 0.5 ºC.

6.2 The Temperature Control System Components

Figure 9 shows the components of the temperature control system. All the elements are described in detail in Appendix E.

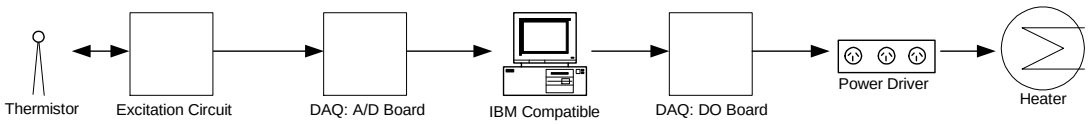


Figure 9 – Temperature control components

The thermistor in conjunction with the excitation circuit produce a voltage signal that is proportional (not linear) to the measured temperature. The voltage signal is digitalised via the DAQ (Data Acquisition) board that is interfaced to the computer. This digitalised-numerical signal is used to calculate the best control signal value. A square wave with a duty cycle proportional to the control signal is used to control the power driver for the heating element. The heater is controlled with a Digital Output (DO) instead of an Analog Output (AO) because of the convenience using a digital on/off power device rather than an analogue power device.

6.3 The Temperature Control System Close Loop

Figure 11 shows the close-loop control used in temperature regulation. Figure 10 is an expansion of the model of the system in which the trend values are considered. This is done because the system identification is considering the linear region around the set point; ie Y(t)=0 if u(t)=0.

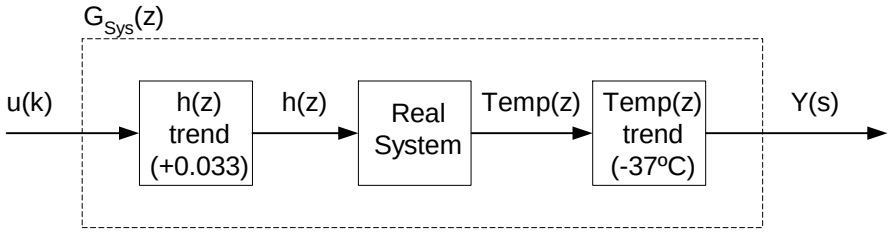


Figure 10 - $G_{Sys}(z)$ relation with the real system

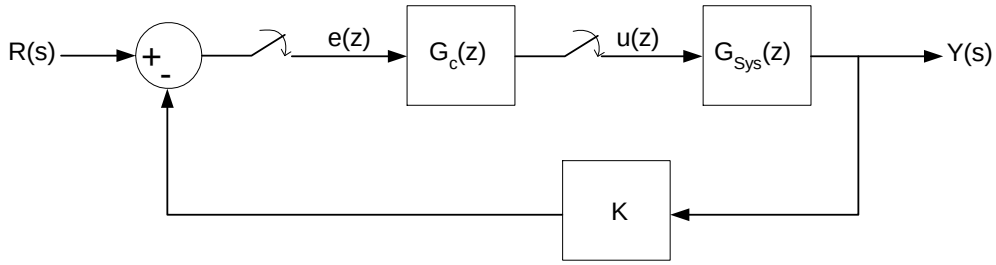


Figure 11 - Close loop control

R(s):	Reference temperature (always 37ºC - 'output-trend*').
e(z):	Sampled error between reference and measured temperature.
u(z):	System control signal + 'input trend*'.
Y(s):	Output (real temperature - 'output-trend*')
$G_C(z)$:	Compensator transfer function.
$G_{Sys}(z)$:	System transfer function.
K	Control constant.
h(z)	Real system input (time averaged power output, using duty cycle method for its variation)
Temp(z)	Real system output.

*: Refer to 6.4 System Identification

6.4 System Identification

The “system” refers to all the elements that have an effect on temperature variation. System Identification is the search of a mathematical model to describe the response of system to a change in a control variable, and is expressed mathematically as a transfer function: $G_{Sys}(z)$. Several steps, described in Table 15, have to be followed to create and validate such models.

Step name	Purpose
Stabilise system	Stabilise the system with 0 input signal (h(t)=0)
Sensibility to h(t)	Determine the sensibility of the output (Temp(t)) to input signal (h(t))
Dominant pole	Determine the stabilising time of the output (Temp(t)) to a step input signal (h(t))
Sampling frequency	Define the best sampling frequency.
System transfer function	Determine the system transfer function
Test transfer function	Evaluate the validity of the transfer function

Table 15 - System Identification Steps

6.4.1 Stabilisation of the System

Without an input signal (h(t)=0), the system runs for a long period of time until the output temperature has stabilised. This accounts for the heat input to the system of other elements such as fan, pumps, etc. when the heater is off (h(t)=0).

6.4.2 Sensibility To h(t)

Determine the sensibility of the output (Temp(t)) to input signal (h(t)). The input signal h(t) is increased by 0.5% steps and left to stabilise. The experiment is stopped when the output temperature is greater than 40ºC, which is a temperature that is not to be reached in a cell expansion situation. Table 16 shows the stabilising temperatures for different input values h(t).

h(t)	Temp($t \rightarrow \infty$)
0.000	26.12
0.005	27.02
0.010	30.54
0.015	32.07
0.020	32.75
0.025	33.76
0.030	36.51
0.035	39.02

Table 16 – Stabilising temperature

6.4.3 Dominant Pole

A time response of the system determines its dominant root. A step function of h(t)=0.033 was used to obtain a final temperature close to 37ºC. Figure 12 shows the step response of the system and its best curve fitting.

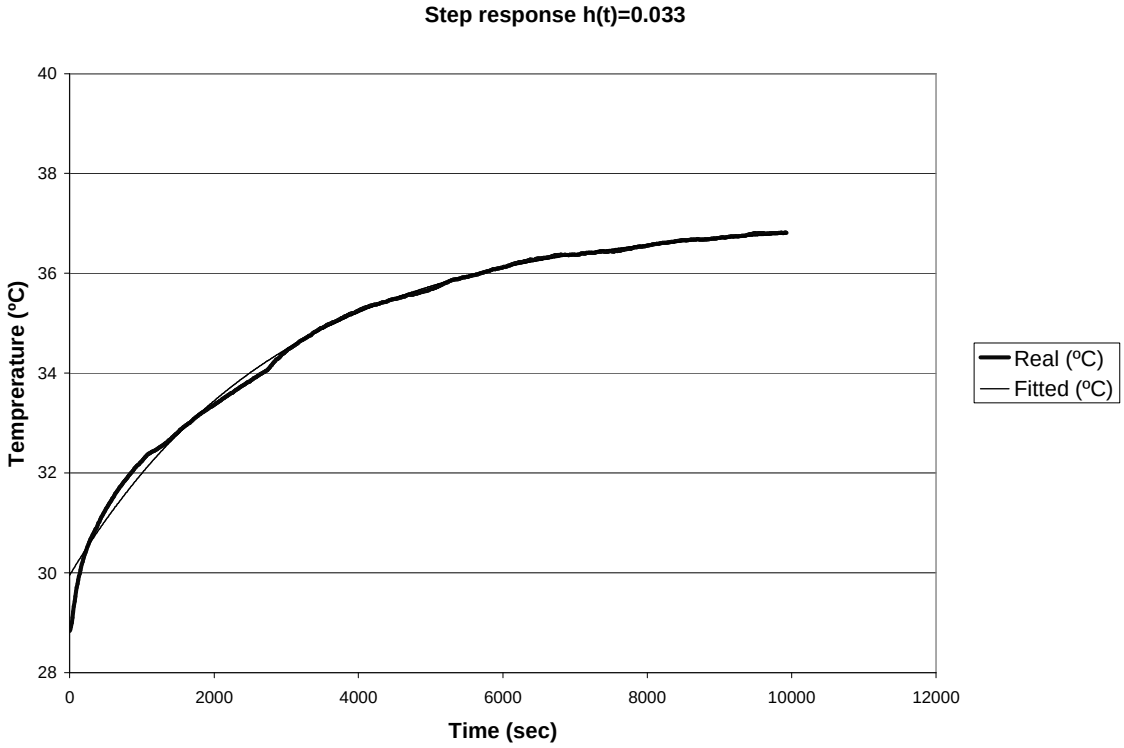


Figure 12 – System step response

The fitted curve (Eq. 10) was calculated using MATLAB (DomPole.m, f.m: Appendix E.5).

$$FittedCurve = 29.9 - (-7.1 * (1 - e^{-\frac{1}{2940\,\mathrm{sec}}t}) \qquad \textbf{Eq. 10}$$

The slowest time constant of the system (τ_{slow} = 2940 sec), which represents its slowest dynamical response, is defined in Eq.10. A minimum time of $3\tau_{slow}$ = 8807 sec = 147 min is required for the system to stabilise within 5% of the steady state value.

6.4.4 Sampling Frequency

The best sampling frequency for the controller is defined from a frequency analysis of the system. Figure 13 show the frequency response of the system for an input of white noise, generated by a uniform random distribution of numbers (with average 0.033), used as the system's input. The power of the frequencies under 0.015 Hz is less than 10dB smaller that the previous peak at 0.008 Hz. Therefore, if the maximum frequency response of the system is considered 0.015 Hz, then a sampling frequency of 0.03 Hz (≈1/30sec) is appropriate (Nyquist theorem). Although in practise, the sampling frequency should be more than 2 times the maximum frequency response, in this case a frequency of 0.03Hz is acceptable, because the highest important frequency component is at about 0.008 Hz.

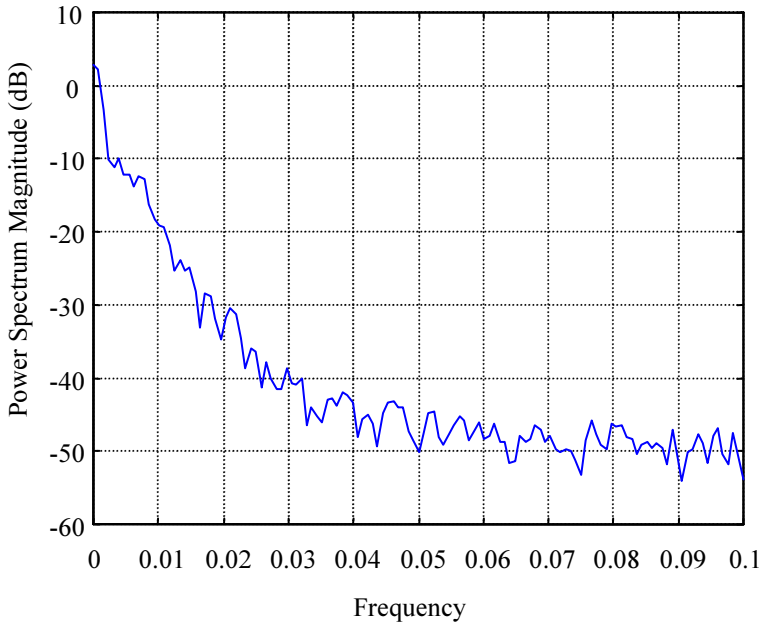


Figure 13 – System frequency response to white noise.

6.4.5 System Transfer Function

Once the two frequency extremes are known (minimum by Dominant pole and maximum by Sampling frequency) and the offset values for input and output are known for an output around 37ºC, then the proper identification is done using a noise input signal. Because white noise contains all frequencies, it is possible to determine the system transfer function by "comparing" the input and the output. This "comparison" is done using the ARX algorithm (30) implemented in the MATLAB identification toolkit (Appendix E.5). Figure 14 show the input and output signals used for the identification (sampling rate 1/30 sec). The system model, with transfer function $G_{Sys}(z)$, is shown in Eq 11.

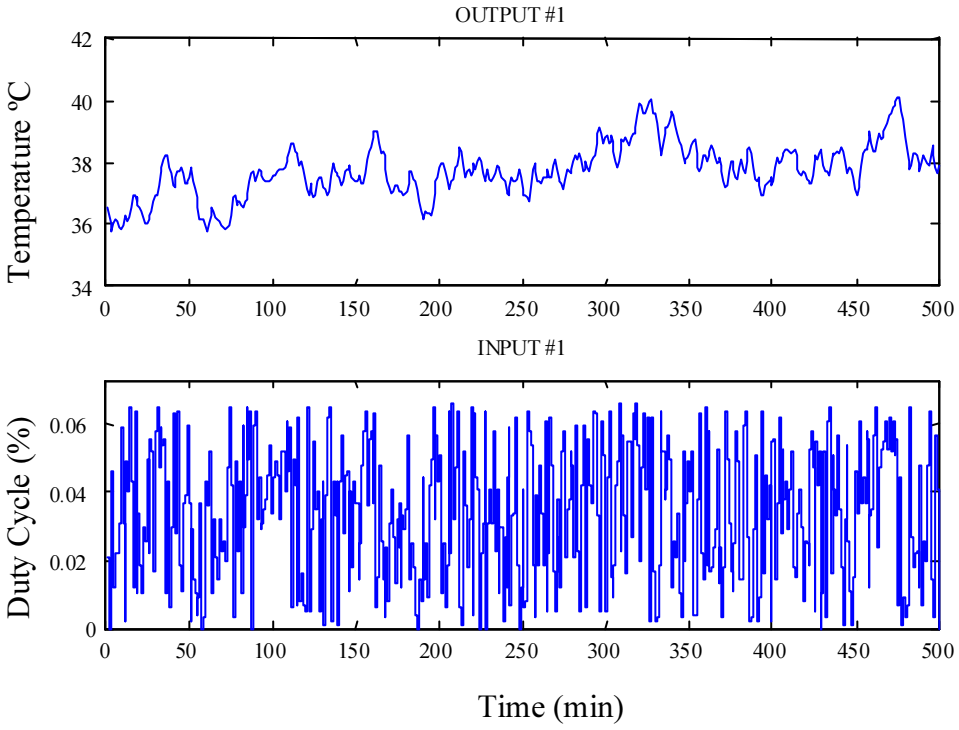


Figure 14 – Input and output signals for system identification

$$G_{Sys}(z) = \frac{3.462z^2 + 7.123z - 5.608}{z^3 - 1.765z^2 + 0.9973z - 0.2265} \qquad \textbf{Eq. 11}$$

6.4.6 Validation Of The Transfer Function

The transfer function is validated comparing the real output of the system with the model's output, for the same input. Figure 15 shows the comparison of the real output and model's output signals.

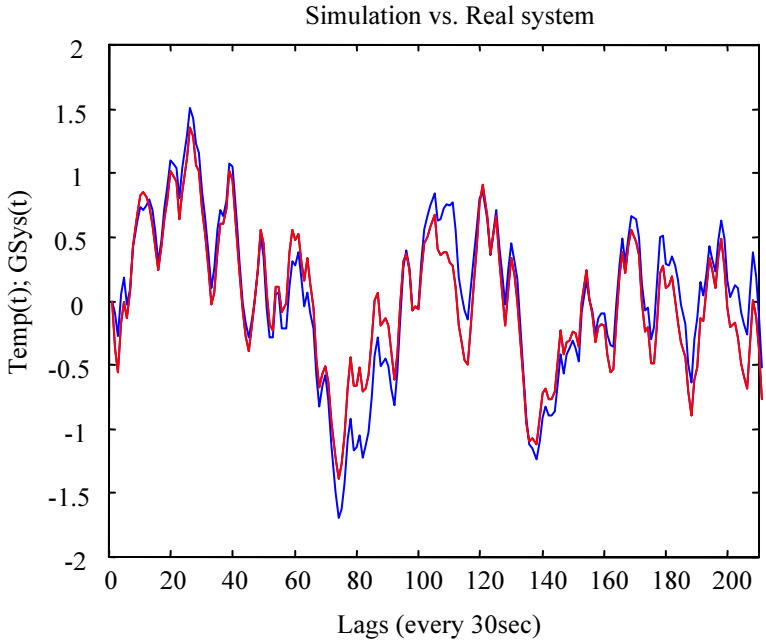


Figure 15 – Simulation vs. Real System

6.5 Compensator Design

The compensator, represented by the transfer function G_C, is the element of the controller that calculates the input signal to the system (e(t)) so that the output variable (Y(t)) follows the reference (R(t)).

The four steps for the compensator design are as follows.

1. Poles and Zeros analysis of $G_{Sys}(z)$

Figure 16 shows the root-locus plot for $G_{Sys}(z)$. By inspection, a very slow pole can be seen, as well as the unstable arms for an increasing K.

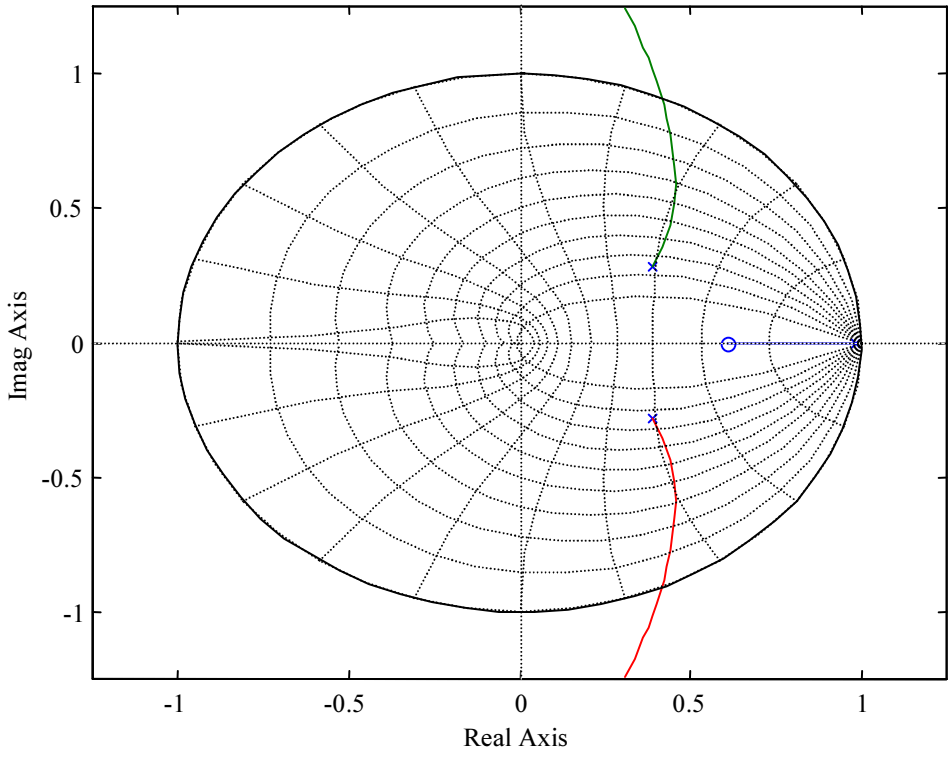


Figure 16 – Root locus for $G_{Sys}(z)$

2. Add integrator to G_C

An integrator block, with transfer function $G_I(z)$, is added to the compensator $G_C(z)$ to ensure zero error for step response. If the difference between the reference (R(t)) and the output (Y(t)) is different than zero, its integration on time will give rise to an increasing error. The compensator uses this error to adjust the input to correct it.

$$G_I(z) = \frac{z}{(z-1)} \qquad \textbf{Eq. 12}$$

3. Poles and Zeros analysis of $G_{Sys}G_C$

G_C a new root-locus diagram (Figure 17) for $G_{Sys}G_C$ (Eq. 13) is obtained when adding new poles and zeros to it. The gain K of the feed back is determined from the root locus diagram.

$$G_{Sys}(z)G_C(z) = \frac{3.4621z(z+2.665)(z-0.6077)(z-0.5)}{(z-0.9866)(z-1)(z+0.65)(z^2-0.7782z+0.2296)} \qquad \textbf{Eq. 13}$$

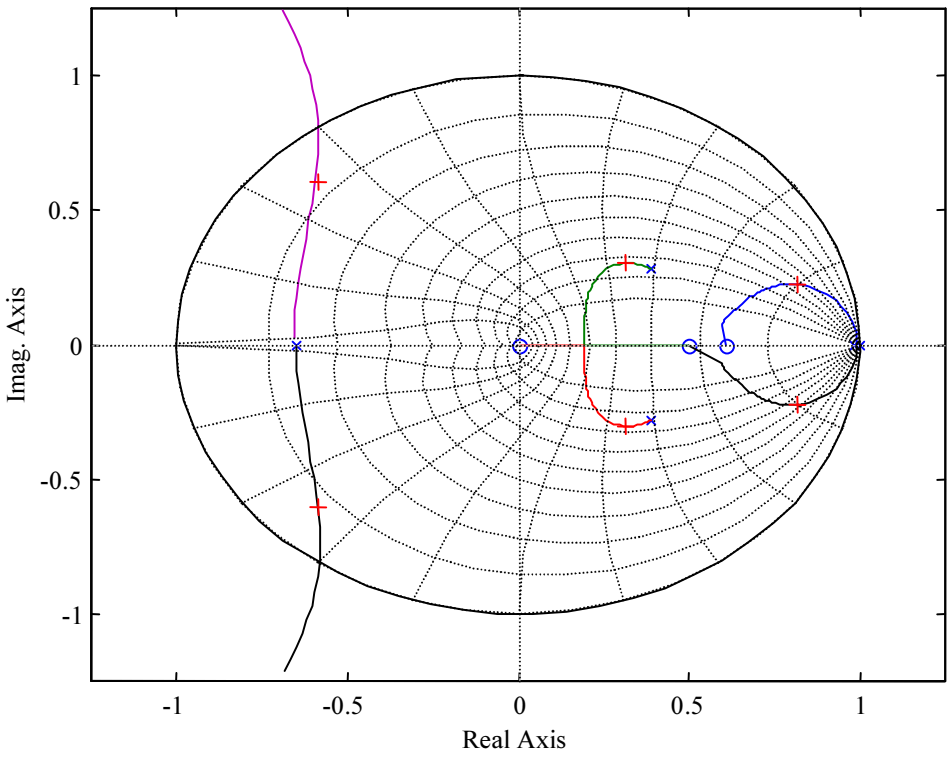


Figure 17 – Root locus for $G_{Sys}(z)G_C(z)$

For the poles shown in the root locus diagram K=0.07.

4. The closed loop

With the system transfer function ($G_{Sys}(z)$), the constant gain K and the control loop system shown in Figure 11 the total transfer function of the system ($G_{Tot}(z)$) can be determine.

$$G_{Tot}(z) = \frac{G_C(z)G_{Sys}(z)}{1 + KG_C(z)G_{Sys}(z)} \quad \textbf{Eq. 14}$$

If

$$G_{Tot}(z) = \frac{N_{Tot}(z)}{D_{Tot}(z)} \quad \textbf{Eq. 15}$$

$$G_{Sys}(z) = \frac{N_{Sys}(z)}{D_{Sys}(z)} \quad \textbf{Eq. 16}$$

$$G_C(z) = \frac{N_C(z)}{D_C(z)} \quad \textbf{Eq. 17}$$

then

$$G_{Tot} = \frac{N_C N_{Sys}}{D_C D_{Sys} + KN_C N_{Sys}} \quad \textbf{Eq. 18}$$

where *N(z)* and *D(z)* are polynomial in *z*. Eq. 18 is used to calculate the expected step response for the controlled system, which is shown in Figure 18. If the expected response is not satisfactory the process of adjusting the compensator G_C goes back to step 3.

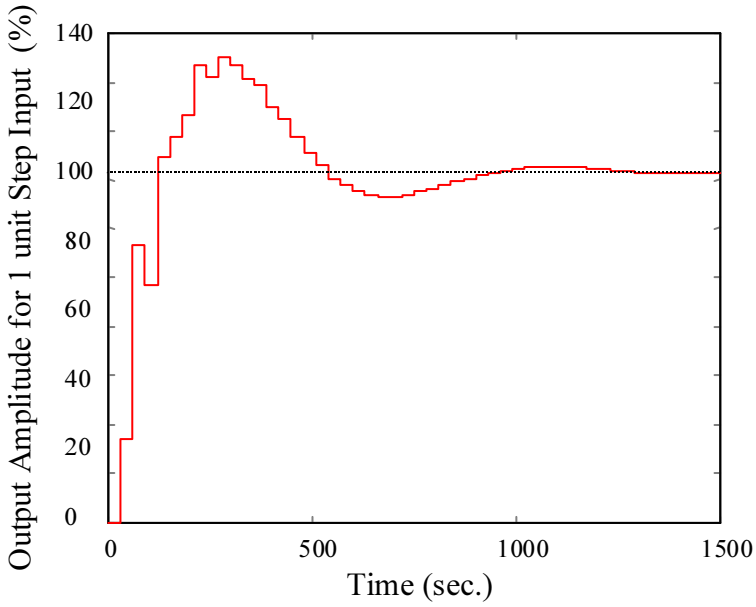


Figure 18 – $G_{Tot}(z)$ Step Response.

Once the compensator is designed and the expected response for the system model and compensator are satisfactory it is experimentally validated.

6.6 Controller Experimental Validation

A simple validation of the model is to compare the its transient response against the transient response of real system to a step input.

Because the model response (Figure 18) and the real system response (Figure 19) are similar in form and values, the model is considered correct. Non linearity and noise in the real system account for the difference between the two responses.

For a complete description of the experiment procedure refer to Appendix B.3.4

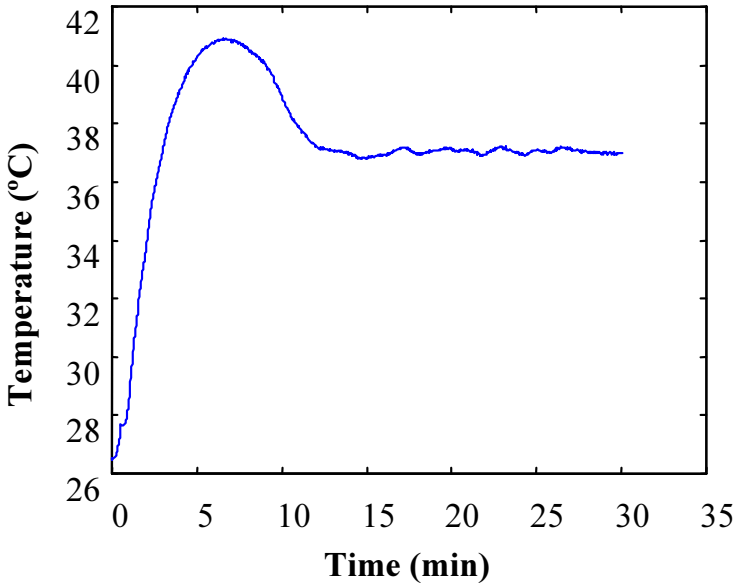


Figure 19 – Experimental Step Response for Real System

The temperature closed loop control provides an illustrative example of the necessary steps in the design of any process-control.

The cell line characterisation, the mass transfer model and the control theory present here provides the basis for development of the prototype system presented in the next chapter.

7 Prototype System Description

This chapter presents the prototype of the intra-capillary hollow fibre bioreactor developed in this project. The physical and functional descriptions of the parts that comprise this system are described below.

The hollow fibre bioreactor, as shown in Figure 20, was implemented and tested. Photo 1 shows the system with the perspex lid removed. Photo 2 is a close-up of the system, in which the hollow fibre module, the media tube, and other components can be seen. The list of components shown in both photos is given in Table 17.

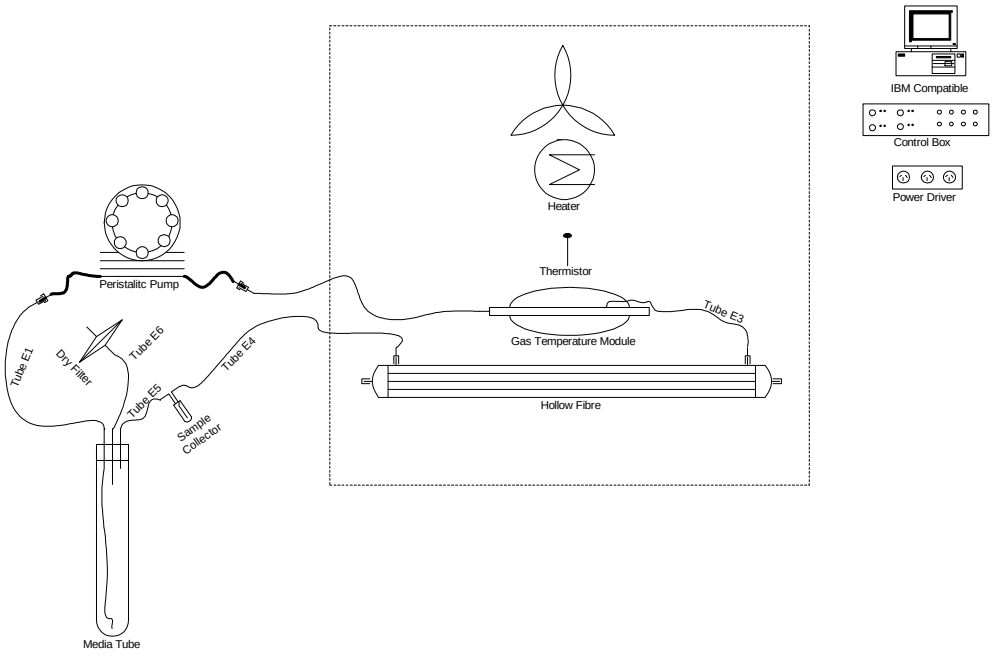


Figure 20 – Prototype System

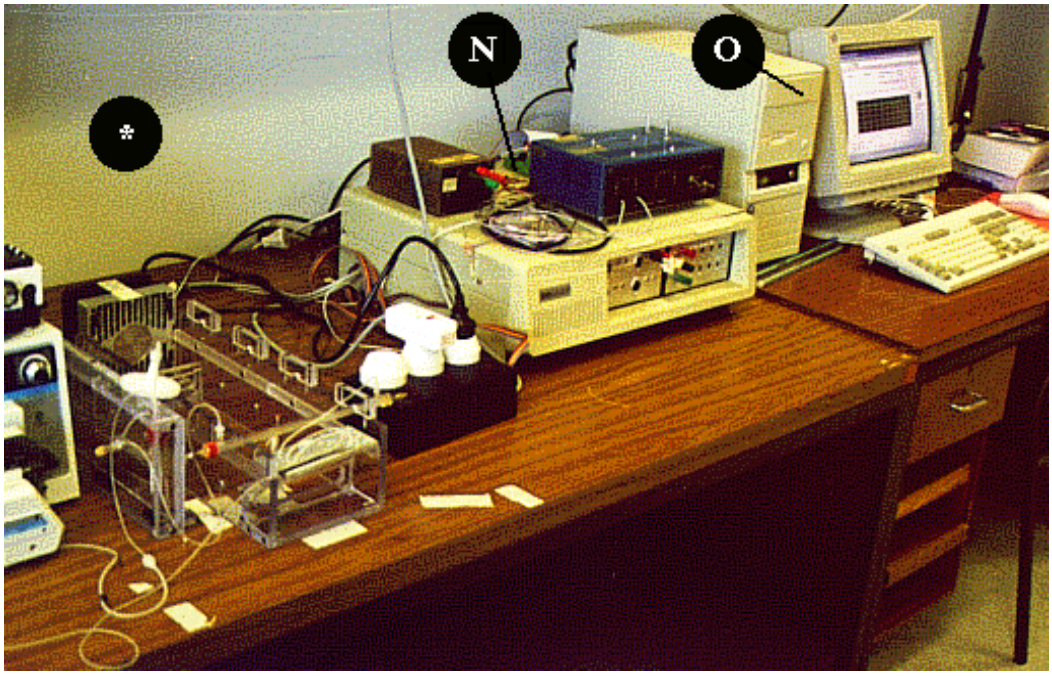


Photo 1 – Prototype System

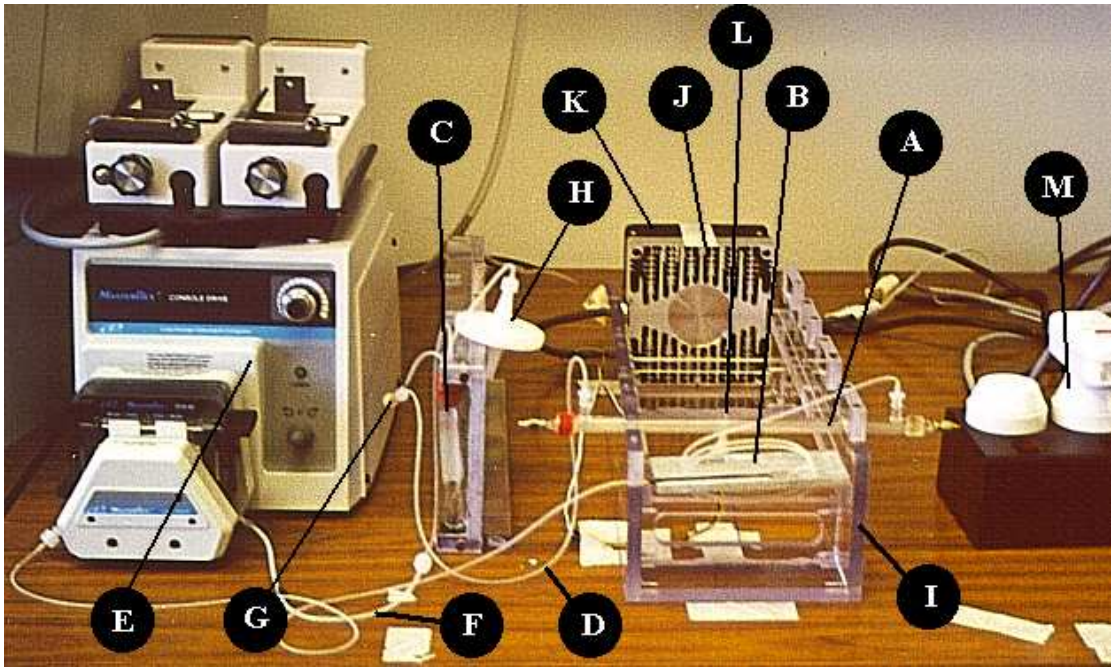


Photo 2 – Prototype System close-up

A. Hollow fibre module	I. Sterilisable base
B. Gas Heat Exchange module	J. Heater
C. Media Tube	K. Fan
D. Silastic Tubing	L. Thermistor
E. Peristaltic pump	M. Power driver
F. Peristaltic tube	N. Control Box
G. Sample collection unit	O. PC computer
H. Dry Filter	* Perspex Lid (removed)

Table 17 – System Parts as shown in Photos 1 and 2

As Figure 20 shows, the intra-capillary perfusion circuit is not implemented in this prototype. All the parts in Table 17 are described in the following sections and a detailed list of components is given in Appendix C.

7.1 Hollow Fibre Module

The hollow fibre module is the core element of the expansion system. The physical and mass transfer characteristics of the hollow fibre module determine the maximum cell density, biomass (cell number) and perfusion requirements.

7.1.1 Physical Description

Figure 21 shows a sketch of a hollow fibre module, which consists of a number of hollow fibres encapsulated in a plastic shell. The internal space of the fibres is interconnected at each end, forming the intra-capillary space. The intra-capillary and extra-capillary spaces are separated by the fibre membrane.

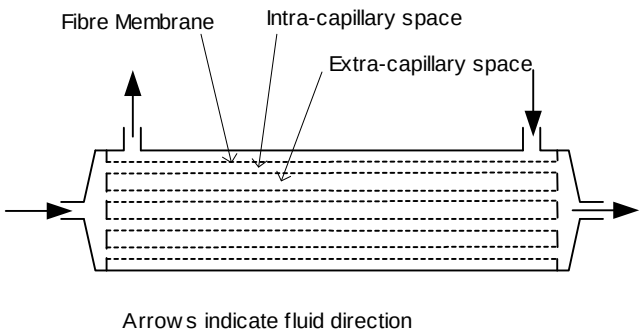


Figure 21 – Hollow Fibre Module sketch

7.1.2 Functional Description

The semipermeable membrane of the fibres allows diffusion of certain molecules across it. When fluids with different concentration of any substance, with MW less than the MW cut-off of the membrane, circulate across the intra-capillary and extra-capillary spaces molecules diffuse and net mass transfer across the membrane occurs.

7.1.3 Hollow Fibre Module Selection

Table 18 shows the parameters that have to be considered in the selection of the hollow fibre module in reference to its physical characteristics.

Hollow Fibre Parameter	Implication
Membrane material	Diffusion properties, molecular weight cut-off, hydraulic permeability.
Fibre length	Surface area; pressure drop along the fibre; max flow rate.
Fibre Diameter	Surface area; pressure drop along the fibre; max flow rate.
Number of fibres	Surface area.

Table 18 – Hollow fibre module parameters & implications.

Other issues to consider in the selection of the hollow fibre module are:

- Availability
- Sterilisation methods and,
- Price

The expansion process is characterised (seeding density, maximum cell density, biomass) after selecting the hollow fibre module. This is the basis for the design or selection of the other parts of the hollow fibre bioreactor.

7.2 Gas And Heat Exchange (GHE) Module

Extra-capillary media gases and temperature is conditioned downstream the hollow fibre module in its flow across the GHE module. This vital component of the bio-reactor is physically and functionally simple, as explained in the next two sections.

7.2.1 Physical Description

Figure 22 shows the components of the GHE module and Table 19 gives a general description of their function

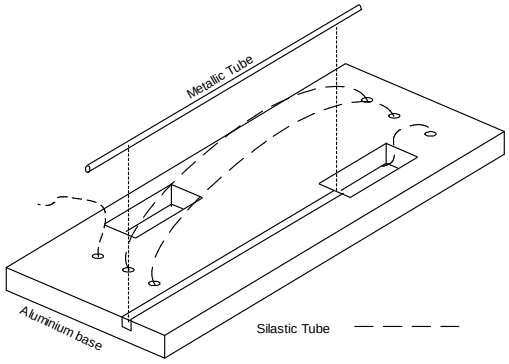


Figure 22 – Gas Heat Exchange Module Components

Component	Function
Grade 313 stain-less steel tube	Media heating.
Metallic base	Conduct heat from surrounds to metallic tube
Silicon tube	Gas transfer and media heating

Table 19– Gas Heat Exchange Module Component & Function

7.2.2 Functional Description

Figure 23 shows the parts of the GHE module. Heat transfer from the environment to the media is done via the metallic base, metallic tube and silastic tube. Gas mass transfer is only via the silastic tube.

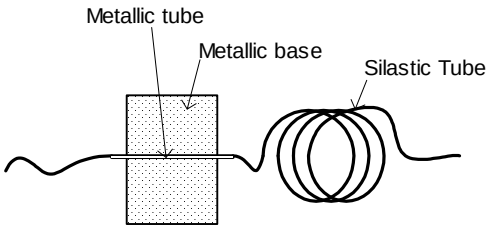


Figure 23 – Gas Heat Exchange Module sketch

The heat and gas transfer characteristics of the silastic tube used were experimentally studied (Section 7.4).

7.2.3 Gas And Heat Exchange Module Design

The parameters considered in the design of the GHE module are

- Maximum media flow rate,
- Gas transfer across the silicon tube,
- Heat transfer across the silicon tube and metallic tube, and
- Use of autoclavable materials for its implementation.

The design is based on mathematical and computational models (Appendix F.3). Because heat transfer obeys the same equations as mass transfer, the same tools were used.

7.3 Media Tube And Dry Filter

The media tube is the media reservoir of the hollow fibre bioreactor. The media tube is vented, to account for volume chances in the system. To prevent contamination a commercial 0.2 μm filter is connected to the ventilation outlet.

7.3.1 Physical Description

Figure 24 shows the media tube and all its parts. This media reservoir is sealed, but has three connection points which are

- input (media return from the hollow fibre module),
- output (media goes to GHE module), and
- vent (filtered air ventilation).

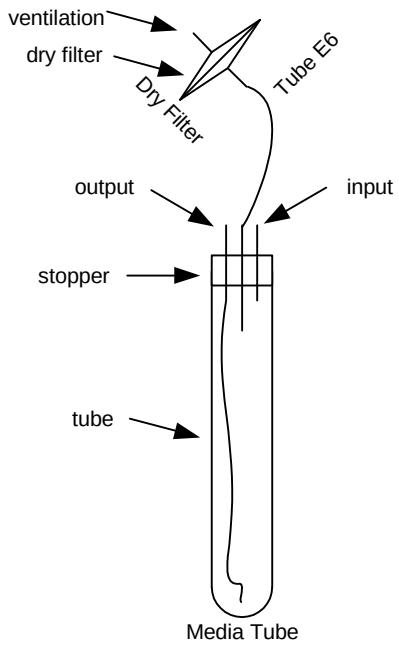


Figure 24 – Media Tube

7.3.2 Functional Description

The three most important functions of the media tube are

- Allows for changes in the extra-capillary media volume resulting from sampling and fluid permeation across the hollow fibre membrane,
- Air trap for circulating bubbles in the system, and
- Allows storage of media at a lower temperature than inside the module.

The media tube is another sterilisable element of the bioreactor, therefore it was implemented with autoclavable materials.

7.4 Silastic Tubing

This autoclavable type of tubing was chosen as the media conduit of the hollow fibre bioreactor, because of its gas exchange characteristics, its availability, and its ease of manipulation.

7.4.1 Silastic Tubing Characteristics

Gas and Heat transfer of silastic tube was experimentally tested. For a detail description of the experiment refer to Appendices B.3.2, B.3.3 (“O2 Transfer in Silastic Tube” and “Heat Transfer in Silastic Tube”.)

The results of the experiments are presented, followed by a discussion of their relevance in the bioreactor design.

Results of Silastic Tubing Characterisation

Figures 25 and 26 show the output temperature and O_2 concentration of H_2O after flowing through a 50 cm silastic tube of ID of 0.04in and OD of 0.085in.

The plots are against 1/flow rate, because this representation is directly related to the time required for fluid saturation. The actual plateau of the graphs is at external temperature and O_2 concentration, respectively.

Temperature reaches its plateau at about 1 min/ml. With flow rate of 1ml/min, a length of 50 cm is necessary for complete temperature conditioning, as shown in Eq. 19.

$$Length(cm) = PlateauTime(\min) * FlowRate(ml/\min) * Length_0(cm) \quad \textbf{Eq. 19}$$

The same is valid for O_2 transfer. The plateau was reached at about 1.5 min, therefore the tubing length necessary to oxygenate media flowing at 1ml/min is 75 cm.

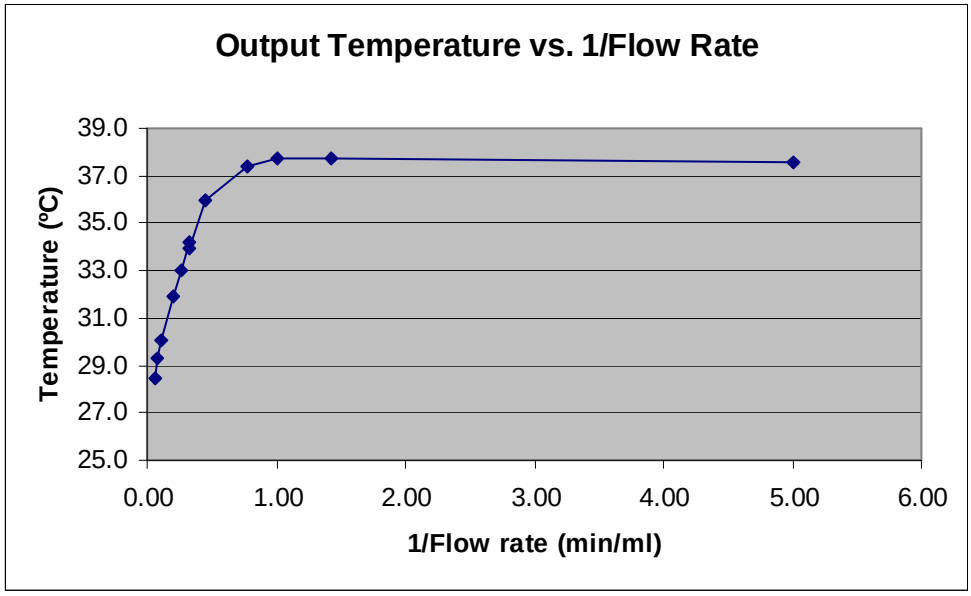


Figure 25 – Temperature vs 1/Flow rate

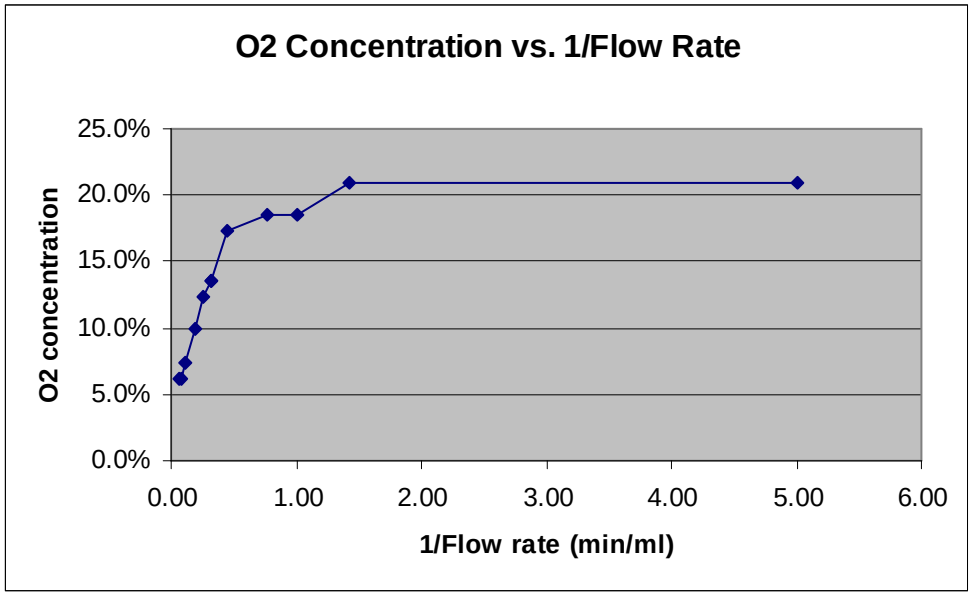


Figure 26 – O_2 concentration vs. 1/ Flow rate

Relevance Of Silastic Tubing In Bioreactor Design

Depending on context good heat and gas transfer of silastic tubing in the bioreactor design can be an advantage or drawback. When silastic tube is used in the GHE module, heat and gas transfer impose a restriction of the minimum amount of tubing, required for the appropriate media conditioning. However, when the silastic tube is used in other parts of the system for media transport, temperature and gas concentrations will follow those of the environment, which are not necessarily close to homeostatic set-points. Therefore, the GHE module has to be placed as close as possible to the hollow fibre module (ie. just upsteam).

Other tube diameters and materials may be appropriate for media transport and their mass transfer properties require evaluation.

7.5 Peristaltic Pump And Peristaltic Tube

The peristaltic pump is the driving force for the extra-capillary media. The flow rate of media is determined by the peristaltic pump setting and the peristaltic tube used. Flow rate for two types of peristaltic tubes and different pump settings were experimentally determined. Figure 27 shows the experimental data and its linear approximations. For experimental details refer to Appendix B.3.1.

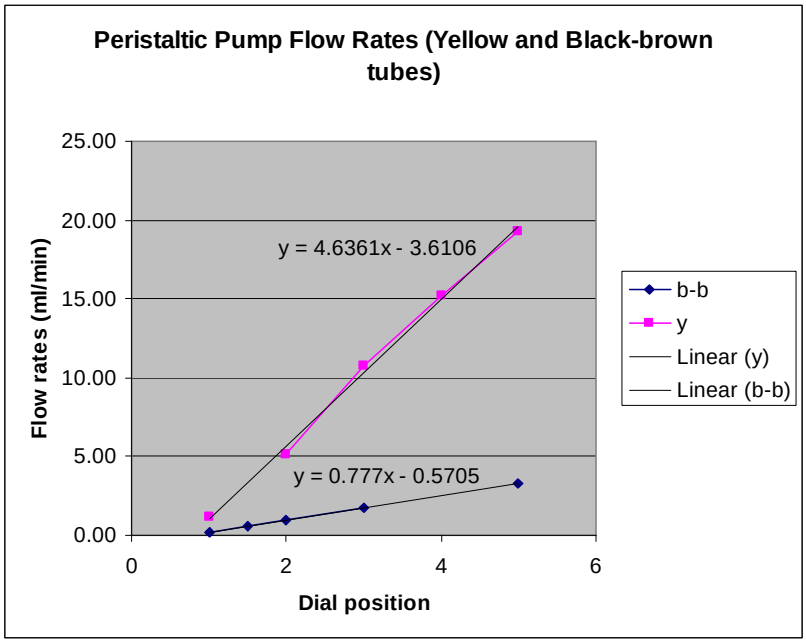


Figure 27 – Peristaltic pump flow rates

The flow rate of another peristaltic tube with different diameter can be determined from the experimental data, if the diameter of the experimental peristaltic tubes is known as shown in Eq. 20

$$NewFlowRate = \frac{NewInternalDiameter}{OldInternalDiameter} OldFlowRate \qquad \textbf{Eq. 20}$$

Once the flow rate is determined and the most appropriate diameter is calculated, the peristaltic tube is selected from a biocompatible and sterilisable material.

7.6 Sample Collection Unit

Samples of the extra-capillary media are taken to evaluate the biomass evolution in the bioreactor. A sample collection unit was developed for this purpose.

7.6.1 Design Of The Sample Collection Unit

The considerations taken for the design of the collector unit were:

- Sterility

- Minimum sampling volume
- Avoid non-circulating media

Figure 28 shows the components of the sample collection unit and flow path of media on it.

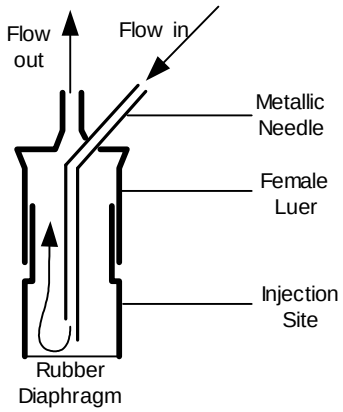


Figure 28 – Sample Collection Unit Components

7.6.2 Functionality Of Sample Collection Unit

The sample collection unit uses an injection site element from which media can be withdrawn through a rubber diaphragm. Media is always circulating inside the sample collector unit, which ensures that there is no dead space and that fresh samples are withdrawn.

7.7 Dry Filter

See Media Tube and Dry Filter, section 7.3

7.8 Sterilisable Base

The sterilisable base, a support for the hollow fibre modules and media tubes, was constructed with autoclavable materials, and can be placed within an autoclave for sterilisation.

7.9 Heater

A commercial air heater was used to maintain the hollow fibre surroundings at a constant temperature. For details of the temperature control system see section 6.2.

7.10 Fan

Air convection was forced inside the confined temperature controlled air region (see Perspex Lid 7.1.16) using a commercial fan.

7.11 Thermistor

A commercial thermistor was used in the temperature control system, because of its ease of use, accuracy, reliability, availability and price. For details of the temperature control system see section 6.2.

7.12 Power Driver

This element was designed to control the heater, a power element, with the computer signal. The power driver opens or closes a power circuit using a TRIAC. The TRIAC is controlled by an optoTRIAC which in turn is controlled by a computer digital signal. The power driver is part of the temperature control system which is described in section 6.2.

7.13 Control Box

For the actual bioreactor prototype, the control box consists only of the excitation circuit for the thermistor (see Appendix C). The control box is part of the temperature control system which is described in section 6.2.

7.14 PC Computer

A commercial IBM compatible personal computer was used as the "intelligent" unit of the control system. A data acquisition board, plugged into the computer, was the element used for its interaction with the other elements of the system. The temperature control logic (see chapter 6) was implemented in LabVIEW. Data logging options were implemented in the same programs.

7.15 Perspex Lid

The perspex lid was used to confine air convection to a space in which temperature was controlled. The lid also fits with the sterilisable frame to form an air tunnel that improves its circulation.

The hollow fibre bioreactor prototype and all its parts were described in this chapter. To complete the description of the prototype system, its operation is presented in the next chapter, illustrated by a cell expansion experiment.

8 Prototype System Operation – Hollow Fibre Bioreactor Expansion Experiment

All the previous chapters set up the basis for cell expansion using the bioreactor prototype developed in this project. Experiments to either characterise the cells or the system (Appendix B) were used in the system design. In this chapter a complete cell expansion process, in which cells and system interact, is described to illustrate the functional characteristics of the hollow fibre bioreactor prototype.

The hollow fibre bioreactor described in chapter 7 was used for the expansion of KG1a cells in RPMI-1640 media. This experiment was used to test the concept and design of the overall developed prototype.

8.1 Aims

Evaluation of the hollow fibre bioreactor prototype by the analysis of KG1a cell growth using glucose and lactate concentration in extra-capillary media as an estimate of biomass.

8.2 Materials And Methods

The expansion process in the hollow fibre bioreactor follows the four main steps of cell culture; cell selection, cell inoculation, cell expansion and harvesting. System sterilisation and sampling are the other two procedures required for cell expansion in the hollow fibre bioreactor prototype. The steps followed in this experiment, which are explained later, are summarised in Table 20.

8.2.1 Cell Selection

KG1a cells grown in RPMI-1640 media where chosen as the cell line to be expanded in the hollow fibre bioreactor. No cell separation was required because all cells came from the same culture in which only one type of cells was present.

Protocol

Step #:	Name:	Date:	Time:	Δ time (h:min):	Qty:	Unit:	Materials:	Action:
1	**Selection of cells**	**Mon-13/07**	**14:00**					**Select the cell line to be expanded**
2	**Sterilise system**	**Mon-13/07**	**14:00**	**1:55**				**Sterilise system**
a	Rig-up pre-sterilise	Mon-13/07	14:00	0:15				
b	Fill with MicroP H2O sterilisation	Mon-13/07	14:15	0:15				
c	Place sterile protection	Mon-13/07	14:30	0:05				
d	Autoclave sterilisation (wet cycle)	Mon-13/07	14:35	1:20				
3	**Cell load**	**Mon-13/07**	**14:00**	**1:15**	**1.0E+07**		**Cells**	**Load cells in bioreactor.**
a	Prepare for cell load (in sterile environment)	Mon-13/07	14:00	0:45				
1	*Rig-up for "washing"*	*Mon-13/07*	*14:00*	*0:15*				
2	*Remove H2O and "wash" with media*	*Mon-13/07*	*14:15*	*0:15*				
3	*Place working media*	*Mon-13/07*	*14:30*	*0:15*				
b	Cell inoculation	Mon-13/07	14:45	0:30				
2	*Rig-up for inoculation*	*Mon-13/07*	*14:45*	*0:15*				
1	*Inoculate cells*	*Mon-13/07*	*15:00*	*0:15*				
4	**Cell grow (Cell expansion)**	**Mon-13/07**	**15:15**	**72:00**				**Configure system for cell grow.**
a	Rig-up for expansion	Mon-13/07	15:15	0:15				
b	Start temperature control	Mon-13/07	15:30	0:15				
c	Start media pumping	Mon-13/07	15:45	0:15				
5	**Sampling**	**Mon-13/07**	**16:00**					**Sampling of extra-capillary media.**
6	**Harvesting**	**Thu-16/07**	**15:15**	**0:30**				**Harvest cells.**
a	Rig-up for harvesting			0:15				
b	"Wash" intra-capillary space in harvesting tube			0:15				

Table 20 - Protocol

8.2.2 Sterilisation

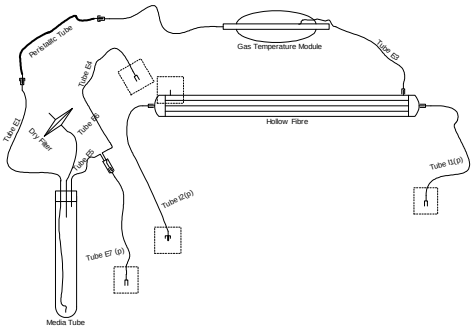
Prior to sterilisation the hollow fibre bioreactor (HBR) is rigged-up as shown in Figure 29 with all the parts mounted in the sterilisable base. Both the intra-capillary and extra-capillary spaces are filled with millipore water. The system is autoclaved (wet cycle) for 25 min. The sterile system is left to cool down and transported to the laminar flow hood for cell inoculation.

8.2.3 Cell Loading – Cell Inoculation

Cell inoculation is the last step in the cell loading process.

The process is simple in concept, but requires multiple steps using sterile procedures to prevent contamination.

The system is rigged-up for washing, as shown in Figure 30. Table 21 shows the necessary steps to remove millipore water from both the intra-capillary and extra-capillary spaces and how the module is prepared with " working" media, used for cell expansion. The extra-capillary space was filled with 7 ml of media.



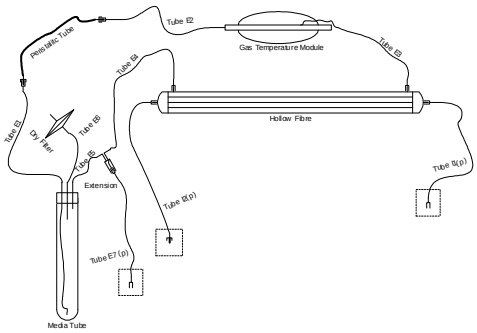


Figure 29 – Pre-sterilisation rig **Figure 30 – Washing rig**

Step #	Action
1	Air Wash intracaplillary space (25ml)
2	Air Wash E7, E5 (close E4)
3	Air Draw E7, E4, E1 (close E5) 25ml
4	Fill Washing media 15ml E7, E4, E1 (close E5)
5	Fill Washing media 1ml E7, E5 (close E4)
6	Fill Washing media 10ml Intracaplilary
7	Draw E7, E5 (close E4) 3ml
8	Draw E7, E4, E1 (close E5) 25ml
9	Air Wash intra 25ml
10	Fill 12 working media
11	Air Wash E7, E5

Table 21 – Preparation for cell inoculation

Prior to cell inoculation, the system is setup, as shown in Figure 31.

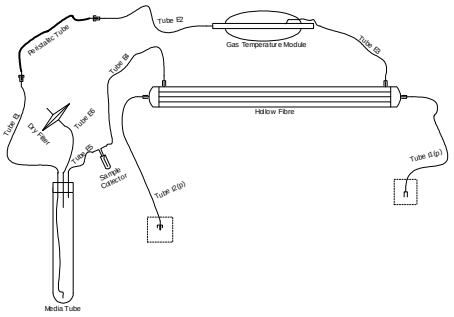


Figure 31 – Cell inoculation rig

The intra-capillary space was washed with 25ml of air with the module in a vertical position to remove any residual fluid. Cells were spun down and suspended in complete media at 1.43e7 cell/ml. 1.2 ml were injected (manually) in the module fol-

lowed by 0.35 ml of air to clear Tube I1(p). The module ends were then closed. The steps for cell inoculation are summarised in Table 22.

Step #	Action
1	Disconet "Extension" with E7 and put "Sampling Plug"
2	Air Wash intra 25 (Vertical)
3	1.2 ml cells
4	0.35 ml air
5	Close Module Ends

Table 22 – Cell inoculation

The total seeding density for the complete bioreactor was 2.47e6 cells/ml as Eq. 21 shows.

$$TotalDensity = icDensity \frac{icVol}{icVol + ecVol} \qquad \textbf{Eq. 21}$$

where

TotalDenisty: Total cell density in the bioreactor,
icDensity: intra-capillary cell density,
icVol: Intra-capillary volume, and
ecVol: Extra-capillary volume.

8.2.4 Cell Expansion

The system is rigged-up for expansion, taken out of the laminar flow hood and placed in the temperature-controlled chamber (which was pre-heated to avoid transients), as shown in Figure 20, chapter 7. The perspex lid placed in position, the peristaltic pump is connected, run at high flow rates (2.5 ml/min) to clear bubbles in the system and finally placed at expansion flow rate (0.6 ml/min). Extra-capillary media samples were taken over the next 24 hours.

Temperature Control

The temperature of the hollow fibre module surrounding environment was maintained at 37 ±0.2 ºC. The thermistor used for temperature measurement was located less than five centimetres downstream from the heated air current.

The temperature was controlled with a PC running LabVIEW® code. The complete close loop temperature control system is explained in detail in chapter 6.

8.2.5 Sampling

Sampling was the most delicate process in terms of sterility, because the system was running in a non-sterile environment. Samples of extra-capillary media (0.5ml) were taken at pre-established times from the sample collection unit with a sterile syringe. The rubber diaphragm was wiped with 70% ethanol. The samples were immediately frozen and glucose and lactate analysis was done 2 days after the experiment was complete. The sampling data is shown in Table 23.

Samples			
Time	Volume (ml)	Lactate (mmol/L)	Glucose (mmol/L)
3/31/98 18:30	0.5	1.76	10.8
3/31/98 19:30	0.5	1.87	10.2
3/31/98 23:26	0.5	3.13	9.95
4/1/98 6:37	0.5	5.86	8.09
4/1/98 10:16	0.5	7.45	7.64
4/1/98 14:40	0.5	8.93	6.71
4/1/98 17:37	0.5	10.2	6.5

Table 23

8.2.6 Cell Harvesting

The system was transported back to the laminar flow hood, where the hollow fibre module was washed with PBS (3 ml) and the cells collected. This was a simple manual process where sterility was not considered.

The total intra-capillary volume recovered was 0.85 ml, because bubbles were present. The total amount of cells recovered was 1.08e8.

8.3 Results

The sampling data is shown in Table 23. The initial total media volume was 8.25 ml. The total time for extra-capillary media turn-over is

$$ecMediaCycleTime = ecVol / ecFlowRate \qquad \textbf{Eq. 22}$$

where

ecMediaCycleTime: Extra-capillary Media turn over,
ecVol: Extra-capillary volume, and
ecFlowRate: Extra-capillary flow rate.

The total number of times that the media recycled (turn-over) between samples ensures that the sample is representative of the total extra-capillary media. Table 24 and 25 show the total number of cycles in between samples (Media cycle #). This is an important issue, because samples are taken upstream the hollow fibre module, before mixing with the rest of the media.

Biomass is calculated from both glucose and lactate concentration in extra-capillary media as follows:

Biomass (cells) = Δ Lactate (mmol/day) / Cell production (mmol/cell/day)
Biomass (cells) = Δ Glucose (mmol/day) / Cell uptake (mmol/cell/day)

The values for cellular lactate production, and cellular glucose specific uptake were experimentally evaluated. In this experiment values for densities of 1e6 cells/ml were used (Glucose Uptake Lactate Production, Appendix B1.1). Tables 24 and 25 show the calculations to estimate biomass using both lactate production and glucose uptake, respectively.

The expected biomass is calculated as follows:

$$Expected\ Biomass = Biomass_0\, e^{t/\tau}$$

which is the well known cell exponential growth with time constant τ.

The Expected Biomass values for the time of sampling is also shown in Tables 24 and 25.

Specific lactate production (mmol/(day*1e6cells)):				0.0024					
Time	**Δ Time**	**Lactate level (mmol/L)**	**Volume (ml)**	**Media Cycle time (min)**	**Media Cycles #**	**Δ Lactate C (µmol/ml/ day)**	**Δ Lactate (mmol/day)**	**Calcualted Biomass (cells)**	**Expected Biomass (cells)**
3/31/98 18:30		1.76	7.75	12:55					1.72E+07
3/31/98 19:30	1:00	1.87	7.25	12:05	4.6	2.64	1.91E-02	7.98E+06	1.76E+07
3/31/98 23:26	3:56	3.13	6.75	11:15	19.5	7.69	5.19E-02	2.16E+07	1.92E+07
4/1/98 6:37	7:11	5.86	6.25	10:25	38.3	9.12	5.70E-02	2.38E+07	2.27E+07
4/1/98 10:16	3:39	7.45	5.75	09:35	21.0	10.45	6.01E-02	2.50E+07	2.47E+07
4/1/98 14:40	4:24	8.93	5.25	08:45	27.5	8.07	4.24E-02	1.77E+07	2.73E+07
4/1/98 17:37	2:57	10.2	4.75	07:55	20.2	10.33	4.91E-02	2.04E+07	2.93E+07

Table 24 – Lactate analysis

Figures 32 is a graphical representation of the calculated and expected biomass for the lactate analysis.

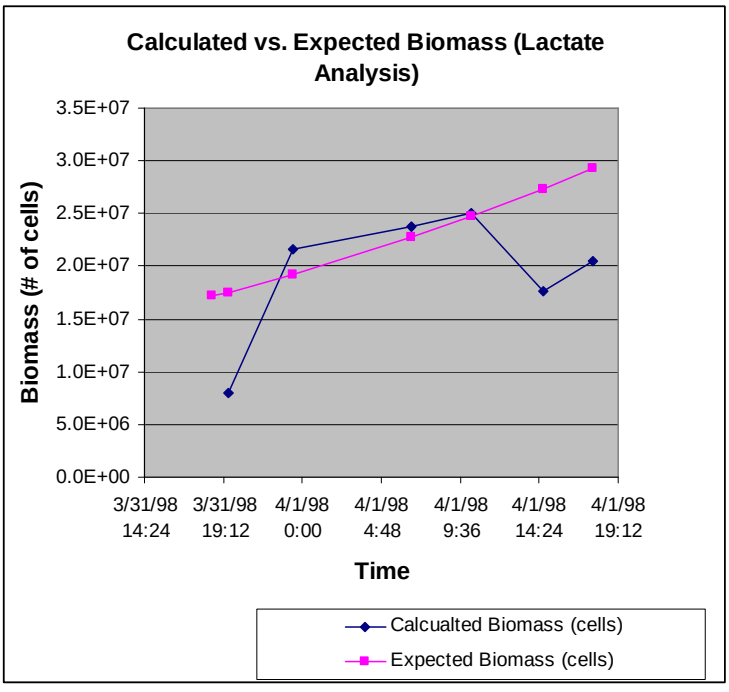


Figure 32 – Lactate analysis

Specific glucose uptake (mmol/(day*1e6cells)):				0.00168					
Time	**Δελτα Τμε**	**Glucose level (mmol/L)**	**Volume (ml)**	**Media Cycle time (min)**	**Media Cycles #**	**Δ glucose C (μmol/ml/ day)**	**Δ glucose (mmol/day)**	**Calcualted Biomass (# cells)**	**Expected Biomass (# cells)**
3/31/98 18:30		10.8	7.75	12:55					1.72E+07
3/31/98 19:30	1:00	10.2	7.25	12:05	4.6	14.40	1.04E-01	6.21E+07	1.76E+07
3/31/98 23:26	3:56	9.95	6.75	11:15	19.5	1.53	1.03E-02	6.13E+06	1.92E+07
4/1/98 6:37	7:11	8.09	6.25	10:25	38.3	6.21	3.88E-02	2.31E+07	2.27E+07
4/1/98 10:16	3:39	7.64	5.75	09:35	21.0	2.96	1.70E-02	1.01E+07	2.47E+07
4/1/98 14:40	4:24	6.71	5.25	08:45	27.5	5.07	2.66E-02	1.59E+07	2.73E+07
4/1/98 17:37	2:57	6.5	4.75	07:55	20.2	1.71	8.12E-03	4.83E+06	2.93E+07

Table 25 – Glucose analysis

Figure 33 is a graphical representation of the calculated and expected biomass for the glucose analysis.

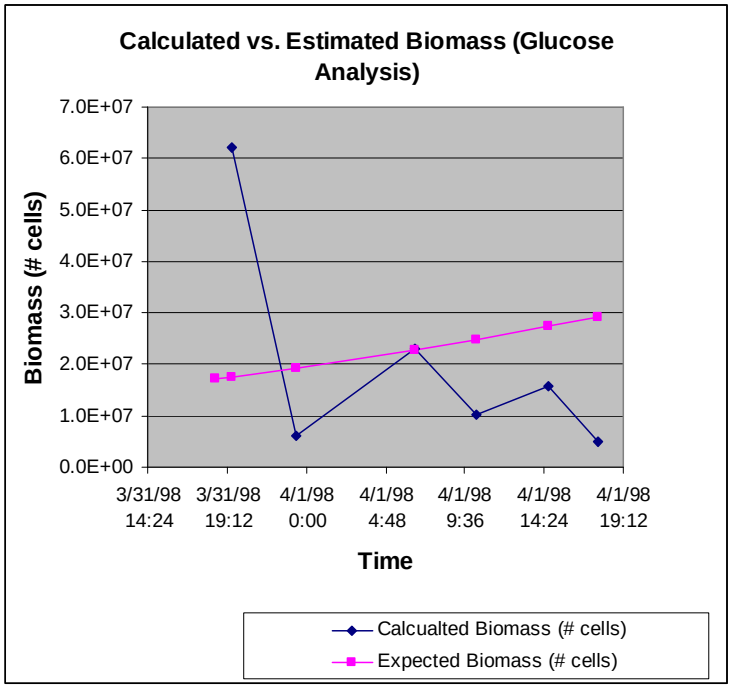


Figure 33 - -Glucose analysis

8.4 Discussion And Conclusions

The concept of the intra-capillary hollow fibre bioreactor was validated, although it is evident that the expansion culture process requires improvement.

Biomass estimation via lactate concentration in extra-capillary media gave close results to the estimated biomass, which was not the case when using glucose concentration.

Biomass decreases at the end of the experiment probably because of the lack of intra-capillary or insufficient extra-capillary perfusion. Molecules with MW greater than the MW cut-off of the hollow fibre membrane where not replenished (if consumed) or cleared (if produced).

The concentration of lactate and glucose of the first media sample is probably not representative for all the media, because samples were taken upstream the hollow fibre module before returning and mixing in the media tube.

Because samples are taken from the extra-capillary space, a lag has to be considered, to account for the diffusion time from intra-capillary to extra-capillary spaces.

A longer experiment with intra-capillary perfusion is the next step in the evaluation of cell growth in the intra-capillary space of the hollow fibre bioreactor.

This chapter completed the development of the intra-capillary hollow fibre bioreactor with its experimental validation. A general discussion that encompasses the complete design and development process, the final prototype and its use is presented in the coming chapter.

9 Discussion

The experimental validation of the hollow fibre bioreactor prototype was the last step in its developing process. In this chapter the design, development and the implementation of the hollow fibre bioreactor prototype are discussed, following the same order in which they were developed during the project.

9.1 System Design And Implementation

9.1.1 Cell Line Characterisation

Cellular metabolic and physical characteristics provide the basis for design and development of the hollow fibre bioreactor. Although a complete understanding of the cells is beyond the scope of this project, it is only necessary to determine critical parameters relevant for the design of the bioreactor.

Intra-capillary and extra-capillary perfusion rates are determined by the cell requirements of substances with MW lower or greater that the cut-off MW of the hollow fibre membrane. O_2, CO_2, lactate and glucose are examples of molecules that diffuse across the hollow fibre membrane. The supply of these molecules depends on both the intra-capillary and extra-capillary perfusion rates.

The production of autocrine growing factors has to be considered to prevent an excessive media perfusion that could "wash" those factors away, and impair cell growth. The opposite case is also true. When autocrine factors inhibit growth, slow media perfusion will not "wash" those factors away and cell growth will be inhibited.

A model of the cells and their interaction with the environment is required for the simulation of the system, and design of appropriate control mechanisms to maintain the best growing conditions during the complete culture process.

An important characteristic of cells, not well studied in this system, is the time it takes for cells to adapt to the new in vitro environment. This latency could include the time it takes for growth factors to activate quiescent cell types or changes in metabolism (aerobic $\rightarrow$ anaerobic).

9.1.2 System Parts

It is preferable to source components “of the shelf” rather than in-house manufacture, however this was not always possible. Component parts of the hollow fibre bioreactor system are divided into two distinctive groups; those that are and those that are not in contact with cells and media. The fundamental properties required of those parts that are in direct contact with the cells and media are sterility and biocompatibility, although sterility also imposes restrictions on the selection of other materials used in the system. For example structural elements which also have to be autoclaved need not be biocompatible.

Of the shelf parts

The hollow fibre module, the most important element of the system, uses the same technology and materials as haemodialysis hollow fibre modules. They have been approved for extra-corporeal therapies, and are not cytotoxic or pyrogenic. The hollow fibre module gives great flexibility to the system. The system is easily scalable by increasing the number of fibres per module. For example a standard renal dialyser has a surface area of $1m^2$, and could theoretically support 10^6 cells/cm^2.

Silicon tube was used for all media transport, because of its biocompatibility, high gas permeability and possibility to be sterilised by autoclaving.

Other of the shelf parts used in the bioreactor are tube connectors, peristaltic pump and other elements used for the in-house manufacture of new parts.

Design of new parts

Design of new parts is an iterative process in which the design is tested and improved on. This process, although slow, gives the opportunity to understand the system in great detail and also gives the flexibility to modify parts where necessary, as was the case for design of the GHE module and sample collection unit.

9.1.3 Computational Tools

The design of a hollow fibre bioreactor is a complex process, in which many factors have to be simultaneously considered. Even though the development of computational tools for the design of the system required significant effort, the insights gained by this design tool would ultimately shorten total development time.

The computational model of diffusion in a cylindrical geometry developed in this project can be used for molecular mass transfer modelling and for heat transfer. The existing model is axisymmetric, but can be expanded to a 3D model to account for non-axisymmetric distributions.

The cell counting methods used for cell growth assays are difficult to use. Accurate methods such as coulter counters and flow cytometry require long set up times, making them impractical when small number of samples are analysed. Estimation of viable biomass is even more difficult and requires the use of indirect measurements such as glucose uptake or lactate production. An improvement on cell counting techniques will be of great benefit for any biotechnology area, and specifically for the assessment of cell culture systems.

9.2 The System

9.2.1 Intra-Capillary Circuit

Intra-capillary perfusion was not implemented in this prototype but the following considerations are relevant for its future development.

The intra-capillary perfusion rate has to be at a level that supplies the appropriate amount of nutrients and high MW molecules, but does not “wash out” cells and autocrine factors. Intra-capillary perfusion has to be adjusted according to the current intra-capillary biomass.

Adding a media sampling mechanism to the intra-capillary circuit will allow a better estimate of biomass.

9.2.2 Extra-Capillary Circuit

The extra-capillary circuit can be improved in many ways. The total volume of media used for the extra-capillary circuit could influence cell growth. If it is too small it will be rapidly depleted of nutrients. On the other hand, a large extra-capillary volume may dilute small autocrine molecules that can cross the hollow fibre membrane (eg. chemokines) and inhibit cell growth.

The media in the extra-capillary circuit could be kept cool to prevent its degradation.

Similar to intra-capillary perfusion, extra-capillary perfusion has to be adjusted according to biomass.

9.2.3 Control Unit

A multi-input multi-output control system will account for interactions between the different variables of the system. Although more complex to realise, it will give a more precise control of the homeostatic environment.

Perfusion

Intra-capillary and extra-capillary perfusion rates have to be adjusted depending on the cell biomass and the concentration of different molecules in media.

Temperature control

Temperature control is of extreme importance. The current system maintains the temperature at a pre-established set point. Some important aspects of temperature control that require future evaluation are:

- Transients

The transient of the heating-up of the system can affect the cells in the hollow fibre module.

- Intra-Capillary Temperature

The intra-capillary temperature was not studied. It was assumed to be constant but that assumption has to be verified.

- Control of Hollow Fibre Module Temperature Versus the Surroundings

The temperature of the air convected around the hollow fibre was controlled, but not of the hollow fibre module itself. A more rigorous control of the temperature within the culture chamber may be achieved by control of the temperature of the media and hollow fibre module. This would require thermistor probes that can be sterilised and inserted in direct contact with media.

O_2 control

The appropriate O_2 concentration is important for cell expansion. This variable was not controlled because the partial pressure of oxygen in air was shown to be adequate for cell growth in flasks. This assumption has to be re-evaluated in future systems, where high cellular densities could decrease O_2 concentration along the hollow fibre module.

CO_2 control

The CO_2 control is important when bicarbonate is used as a pH buffer. Most bicarbonate buffering systems require a CO_2 concentration between 5-10% (V/V). The conditioning of media with CO_2 can be achieved using the same gas exchange elements as for O_2 (Silastic tubing) with the modification that the gas exchange tubing would be placed within a CO_2 gas-controlled chamber.

Non-linear control

A non-linear control system would improve the transient response of the system because of its inherent non-linear characteristics. However a more complicated control system would take longer to design, and the extra-effort may not be justified in terms of improved performance.

9.3 System Operation

The use of the hollow fibre bioreactor is simple to understand; however, because multiple steps are required to load, expand and harvest cells, the process is complex and requires automation. The most critical aspect of system operation is sterility because a break in the sterility can easily lead to contamination. It would be preferable to assemble the system before autoclaving rather than assembling sterile parts inside a laminar flow hood.

9.3.1 Separation

Cell separation can be performed in the same hollow fibre module and is described elsewhere (14). Coupling the separation process to cell expansion was not examined and would require development of additional software and hardware.

9.3.2 Inoculation

The efficiency of inoculation will be reflected in the final biomass of expanded cells. Cells that are present in the headers of the hollow fibre module will undergo necrosis. Dead volumes in the inoculation circuit are considerable with respect to the total intra-capillary space volume. Automation of the inoculation process as described in reference (14) will greatly improve system performance.

The efficiency in the actual inoculation process is low, which is a great drawback when high yields are required. This is due to the manual inoculation technique, but an automated procedure will help resolve this problems in future developments

9.3.3 Expansion

The expansion process, the central function of the hollow fibre bioreactor, is affected by any change of in control parameters or loading procedures. The actual perfusion systems allow cells to be maintained without a break in sterility and cell “feeding” is automated. In future bioreactor systems the expansion process has to be optimised by “fine tuning” of system control parameters such as perfusion rates, temperature control, gas transfer and so on.

9.3.4 Harvesting

Harvesting is generally a straightforward process and only involves flushing the intra-capillary space with media, if cells are not adhering to hollow fibre membranes.

9.3.5 Sampling

Sterility was the most critical issue during the sampling process. This process is not automated at all, which makes the system “operator dependent”. Because measurements were not taken in line, media has to be frozen after sampling.
The automation of the sample mechanism will be of great advantage for its operation as well as for the prevention of contamination.

10 Conclusions

The intra-capillary hollow fibre bioreactor is a good alternative as a cell expansion device, because of its efficient mass transfer characteristics. This allows a homogeneous supply and control of cells' microenvironment and the possibility of integration of a cell separation system using the same hollow fibre module.

The design and implementation of a hollow fibre bioreactor requires a good understanding of many different fields, such as mass transfer, control theory, and cell biology, in addition to sterile cell handling techniques.

The cell counting methods (grid cytometry, flow cytometry) used during this project, are either slow or difficult to use. Grid cytometry is inaccurate, and flow cytometry requires a long set up time, which makes it impractical when only a few samples are analysed.

The use of mathematical and computational models of the system is essential for its design. The computational model developed, although very "expensive" in computer capacity, is flexible and easy to use. The investment in time for its development is worth all of the future benefits.

The expected final biomass and the characteristics of the cultured cell line define the requirements for the design and development of the system. Physical characteristics of the cell impose restrictions in the volume and size of the hollow fibre. Cell metabolic characteristics impose restrictions in respect of mass transfer, perfusion and control requirements.

Cell characteristics, that impose technical implications in the development of the bioreactor, need to be re-evaluated, as was the case with variability of cell specific uptake with respect to seeding density.

Sterility affects all the steps of design and construction of the bioreactor and places restrictions on system implementation.

The temperature linear control system worked fine. Even though temperature is an easy variable to work with, the development of the control system shows the difficulties of a real-time control implementation.

The system did provide small MW molecules (less then hollow fibre MW cut-off), but large MW molecules were not provided, because of the lack of intra-capillary perfusion.

The system is not completely automated, making it difficult to use. It has to be autoclaved and setup for cell inoculation under sterile conditions, for which a complicated protocol has to be followed. Sampling is susceptible to contamination and requires the presence of an operator.

Despite these initial technical difficulties, this prototype gives a good theoretical and technical basis for the design and development of an intra-capillary hollow fibre bioreactor that conforms to pharmaceutical standards of GMP and is commercially feasible.

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Appendix A - CD Structure

The structure of the included CD is presented below. The structure of the appendices in the document is replicated in the structure of the appendices files (`...\Project\Appendices...`) included in the CD.

```
...\Project\Appendices        \CD Structure
                              \Experiments   \Cells
                                             \Cells and System
                                             \System
                              \System Parts
                              \System Software\TempControl     \Data
                                                               \SubVI
                              \Tools         \Cell counting software
                                             \Cell Handling
                                             \HF Mass Trans Algebraic
                                             \HF Mass Trans Computational Model
                                                               \Data
                                                               \SubFiles
                                                               \SubFunctions
                                             \System Identification
                                             \Visio
          \Document
          \Document Inserts   \Excel
                              \Microsoft Photo Editor
                              \Visio
```

Subdirectories structure of included CD

Appendix B - Experiments

All the experiments include a template (in the included CD, *.dot) for future repetitions.
The experiment description, protocol, tables for data entry, analysis and results are done in an EXCEL workbook; and all the experiments have the same general structure.

All the experiments are set up in an EXCEL workbook, with the following pages (at least):

- Aims and Description
- Protocol
- Data
- Analysis
- Results

Other work sheets are added for specific experiments, to make it clear how to set up the experiment, and to help to organise the timing and scheduling of an experiment. Some examples are:

- Setup
- Time
- Dilution

The experiments are divided in three types which are:

- Cells alone
- Cells and System
- System alone

B.1. Cells Experiments

B.1.1 Glucose Uptake Lactate Production

Glucose uptake and lactate production are important variables in cell metabolism. These variables are to be used as cell biomass estimators in the hollow fibre bioreactor during the expansion process.

Aims

Evaluate the specific glucose uptake and lactate production in KG1a cells cultured in RPMI-1640 media for different cell concentrations.

Material and Methods

KG1a cells were cultured in suspension (RPMI-1640) at 6 different cell densities (see Table B.1.1 – 1) in 12 well dish plates with a volume of 1ml per well.

CODE:	Cell dens. (cell/ml):
Media	0.00E+00
1A	1.00E+05
1B	1.58E+05
1C	2.51E+05
2A	3.98E+05
2B	6.31E+05
2C	1.00E+06

Table B.1.1 - 2

The experiment was done by quadruplicate. For the three lower densities, samples were taken after 49:00 and 73:00 hours. For the three higher densities the samples were taken after 24:00 and 48:00 hours. Cell density was measured for each sample. After cell spinning, media was frozen for future measurement of glucose and lactate concentration. The protocol used for this experiment is summarized in Table B.1.1 – 2.

Protocol

#:	Name:	Date:	Time:	Qty:		Unit:	Materials:	Action:
1a	Create Samples	Tue-12/05	16:00	4 x 6.50E+06 =		#	Cells	Prepare cell cultures (2.5ml) at 6 different densities (2C, 2B, 2A, 1C, 1B, 1A) from 4 different cell sources (I, II, III, IV).
				4 x 15.39=	61.56	ml	Media	
1b					4	#	12 well dish	Fill in 12 well dishes (1ml each well) (See 12 Dishes, Samples).
1c				2 x 5=	10	ml	Media	Make Vile Tube Media Samples (See 12 Dishes, Samples).
1d								Froze India
2	Alfa & Eco Samples	Wed-13/05	10:00		12	#	Vile Tubes	Take the samples from Alfa, centrifuge it and froze media. Froze Eco.
3	Beta & Fochstrod Samples	Thu-14/05	10:00		12	#	Vile Tubes	Take the samples from Bravo, centrifuge it and froze media. Froze Fochtrod
4	Charlie & Golf Samples	Thu-14/05	10:00		12	#	Vile Tubes	Take the samples from Charlie, centrifuge it and froze media. Froze Golf
5	Delta & Hotel Samples	Fri-15/05	16:30		12	#	Vile Tubes	Take the samples from Delta, centrifuge it and froze media. Froze Hotel

Table B.1.1 - 2

The cellular specific uptake or production was calculated as follows.

$$Uptake = -\frac{\Delta C}{\Delta t \cdot CellDensity}$$

where

Uptake: Specific uptake of any substance,

ΔC: Change in substance concentration in Δt, and

CellDensity: Logarithmic average of cell density at t=0 and t=Δt.

Table B.1.1 – 3 and B.1.1 – 4 show the acquired data and its analysis.

1

Date & Time
19-May 14:00

India		
Sample	**Lactate**	**Glucose**
mean	1.805	11.850
sdtv	0.064	0.354

3

Date & Time
21-May 14:00

Foxtrot		
Sample	**Lactate**	**Glucose**
mean	1.785	11.600
sdtv	0.021	0.141

Bravo				t0 -> t2							
Sample	**Cell density (cell/ml)**	**Lactate**	**Gluc.**	**Density log Avg (cell/ml)**	**Δt (h)**	**Δ Lactate (mmol/L)**	**Lactate prod. (mmol/cell/hour)**	**Δ Glucose (mmol/L)**	**Glucose uptake (mmol/cell/hour)**	**Lact. Avg & Stdev**	**Gluc. Avg & Stdev**
2A-I	1.4E+06	6.18	6.63	8.00E+05	48:00	4.375	1.1E-10	-5.220	1.4E-10	1.3E-10	1.2E-10
2A-II	1.5E+06	7.07	7.68	8.34E+05	48:00	5.265	1.3E-10	-4.170	1.0E-10	9%	18%
2A-III	1.5E+06	7.38	8	8.14E+05	48:00	5.575	1.4E-10	-3.850	9.9E-11		
2A-IV	1.2E+06	6.36	6.98	7.16E+05	48:00	4.555	1.3E-10	-4.870	1.4E-10		
2B-I	2.4E+06	7.2	5.59	1.33E+06	48:00	5.395	8.4E-11	-6.260	9.8E-11	9.4E-11	8.9E-11
2B-II	2.2E+06	8.78	6.98	1.26E+06	48:00	6.975	1.2E-10	-4.870	8.0E-11	17%	8%
2B-III	2.3E+06	7.82	6.17	1.28E+06	48:00	6.015	9.8E-11	-5.680	9.2E-11		
2B-IV	2.7E+06	7.3	5.87	1.43E+06	48:00	5.495	8.0E-11	-5.980	8.7E-11		
2C-I	3.8E+06	11.1	4.73	2.08E+06	48:00	9.295	9.3E-11	-7.120	7.1E-11	9.8E-11	7.2E-11
2C-II	2.8E+06	9.81	4.4	1.74E+06	48:00	8.005	9.6E-11	-7.450	8.9E-11	8%	20%
2C-III	3.2E+06	11.8	5.33	1.91E+06	48:00	9.995	1.1E-10	-6.520	7.1E-11		
2C-IV	4.3E+06	12	5.87	2.27E+06	48:00	10.195	9.3E-11	-5.980	5.5E-11		

Table B.1.1 – 3 – Analysis for samples 2A, 2B and 2C

5	Date & Time											
	22-May 15:00											
	Hotel											
	Sample	Lactate	Glucose									
	mean	1.715	11.250									
	sdtv	0.064	0.495									
	Delta				t0 -> t2							
	Sample	Cell density (cell/ml)	Lactate	Gluc.	Density log Avg (cell/ml)	Δt (h)	Δ Lactate (mmol/L)	Lactate prod. (mmol/cell/hour)	Δ Glucose (mmol/L)	Glucose uptake (mmol/cell/hour)	Lact. Avg & Stdev	Gluc. Avg & Stdev
	1A-I	6.6E+05	5.81	9.89	2.97E+05	73:00	4.005	1.8E-10	-1.960	9.0E-11	1.7E-10	1.1E-10
	1A-II	6.7E+05	5.62	9.42	3.00E+05	73:00	3.815	1.7E-10	-2.430	1.1E-10	6%	11%
	1A-III	7.2E+05	5.49	9.16	3.14E+05	73:00	3.685	1.6E-10	-2.690	1.2E-10		
	1A-IV	6.9E+05	5.63	9.38	3.05E+05	73:00	3.825	1.7E-10	-2.470	1.1E-10		
	1B-I	1.3E+06	7.51	7.94	5.51E+05	73:00	5.705	1.4E-10	-3.910	9.7E-11	1.6E-10	1.1E-10
	1B-II	1.0E+06	7.45	7.99	4.69E+05	73:00	5.645	1.7E-10	-3.860	1.1E-10	6%	8%
	1B-III	1.1E+06	7.29	7.82	4.74E+05	73:00	5.485	1.6E-10	-4.030	1.2E-10		
	1B-IV	1.1E+06	7.39	8.06	4.77E+05	73:00	5.585	1.6E-10	-3.790	1.1E-10		
	1C-I	1.7E+06	9.6	6.47	7.55E+05	73:00	7.795	1.4E-10	-5.380	9.8E-11	1.4E-10	9.7E-11
	1C-II	1.7E+06	9.79	6.55	7.66E+05	73:00	7.985	1.4E-10	-5.300	9.5E-11	2%	2%
	1C-III	1.7E+06	9.57	6.39	7.55E+05	73:00	7.765	1.4E-10	-5.460	9.9E-11		
	1C-IV	1.6E+06	9.7	6.62	7.28E+05	73:00	7.895	1.5E-10	-5.230	9.8E-11		

Table B.1.1 - 4– Analysis for samples 1A, 1B and 1C

Results

Figures B.1.1 – 1 and B.1.1 – 2 show lactate production and glucose specific uptake of KG1a cells in RPMI-1640 media, respect to cell density.

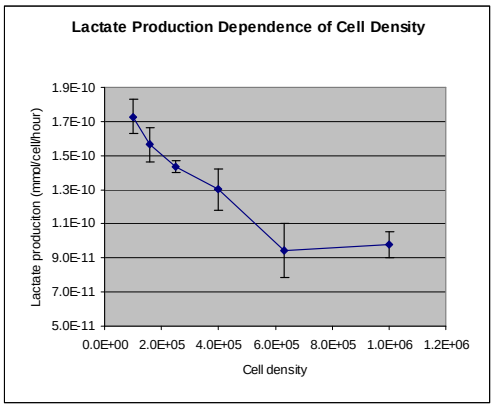


Figure B.1.1 – 1

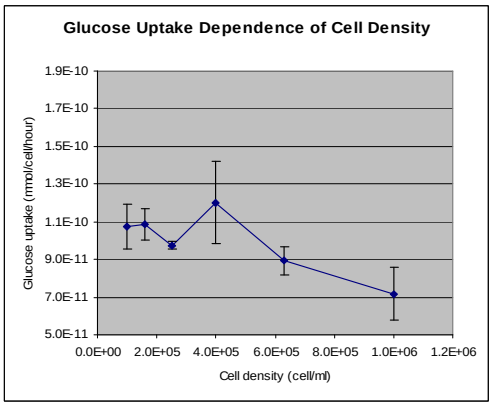


Figure B1.1 – 2

Discussion and Conclusions

Lactate production and glucose specific uptake of KG1a cells grown in RPMI-1640 media is dependent on cell density. Both, lactate production and glucose uptake, decrease for an increase in cell density. This represents a decrease in cell metabolism. Lactate production and glucose uptake do not vary in the same manner. This could represent a change from aerobic to anaerobic metabolism.

B.1.2 Media Aging

The effect of media aging in cell growth, at cell culture temperature, is relevant in the hollow fibre bioreactor design, because a cell expansion process could last many days.

Aims

Evaluate the effect of media (RPMI-1640) ageing at 37ºC in cells (KG1a) growth.

Material and Methods

Six media samples (6ml each), from the same batch, are frozen. Every third day one sample is unfrozen and placed in the incubator at 37ºC. The day in which the last sample is unfrozen, a sample (0.5 ml) of each is taken for glucose and lactate concentration analysis. KG1a are cultured in the remaining media for 4 days, and cell density is measured the first, second and forth days. Table B.1.2 – 1 summarizes the protocol used for this experiment.

Protocol

#:	Name:	Date:	Time:	Qty:	Unit:	Materials:	Action:
1	Make media samples & freeze them.	Fri-8/05	10:00	36	ml	Media	Make 6 media samples M1-M6 (6ml each) and freeze them.
2	Unfreeze M1, place at 37ºC	Sun-10/05	10:00				
3	Unfreeze M2, place at 37ºC	Wed-13/05	10:00				
4	Unfreeze M3, place at 37ºC	Sat-16/05	10:00				
5	Unfreeze M4, place at 37ºC	Tue-19/05	10:00				
6	Unfreeze M5, place at 37ºC	Fri-22/05	10:00				
7	Unfreeze M6, place at 37ºC	Mon-25/05	10:00				
8a	Glucose & lactate samples.	Mon-25/05	10:00	6		Vile tubes	Collect samples (0.5ml, Vile tube) for glucose and lactate measurements.
8b	Make cell cultures (KG1a)			(1.5e+5)*5*6 =	4.50E+06	Cells	Make 6 cell cultures C1-C6 with media M1-M6 with a seeding density of 1.5e+5 cells/ml.
8c	Seeding density						Count cells in cultures C1-C6
9	Cell count - 1 day	Tue-26/05	10:00				Count cells in cultures C1-C6
10	Cell count - 2 day	Wed-27/05	10:00				Count cells in cultures C1-C6
11	Cell count - 4 day	Fri-29/05	10:00				Count cells in cultures C1-C6

Table B.1.2 – 1 – Media Aging Protocol

Results

Glucose concentration, lactate concentration and final cell density, related to media age, are shown in Table B.1.2 – 2.

Media Age (days)	Glucose (mmol/L)	Lactate (mmol/L)	Final Cell Density (cells/ml)
0	11.6	1.73	1.40E+06
3	11.2	1.68	1.24E+06
6	11.3	1.71	1.11E+06
9	11.3	1.73	1.20E+06
12	9.53	1.52	1.07E+06
15	5.76	0.88	9.90E+05

Table B.1.2 – 2 – Media Aging effects.

Discussion and Conclusions

Media characteristics change when stored at 37ºC. Some possible variations, which have a negative effect in cell growth, are

- reduction in concentration of glucose,
- degradation of growth factors in FCS, and
- degradation of amino acids (such as glutamine).

This experiment confirmed the reduction in glucose and lactate concentrations. Other parameters that affect cell growth have to be evaluated in future experiments.

B.1.3 Seeding Density Growth Dependence

The effect of seeding density in cell growth is a parameter that has to be considered in the design of the hollow fibre bioreactor. Because cells reside in the intra-capillary space (only), cell density in the complete system (including intra-capillary and extra-capillary media) is a fraction of the initial seeding concentration.

Aims

Determine how growth of KG1a cells in RPMI-1640 media depends on seeding density.

Material and Methods

KG1a cells are cultured in RPMI-1640 media at six different densities, as shown in Table B.1.3 – 1.

CODE:	Cell density (cell/ml):
1	1.00E+03
2	2.51E+03
3	6.31E+03
4	1.58E+04
5	3.98E+04
6	1.00E+05

Table B.1.3 – 1 – Seeding densities

Cells are cultured for seven days and cell densities are measured the first, second, forth and seventh days. Table B.1.3 – 2 summarises the protocol used in this experiment.

Protocol

Step #:	*Name:*	*Date:*	*Time:*	*Qty:*	*Unit:*	*Materials:*	*Action:*
1a	Create samples	Tue-12/05	14:00	1.68E+06		cells	Create 3 sets (A,B,C) of 6 samples (0.5ml each, 1,2,3,4,5,6) of different seeding densities (see Samples), and place in 24 well dishes (Alfa, Bravo, Charlie & Delta)
1b	Cell densities seeding						Cell density measurement.
2	Cell densities 1st day	Wed-13/05	14:00				Cell density measurement (Alfa)
3	Cell densities 2nd day	Thu-14/05	14:00				Cell density measurement (Bravo)
4	Cell densities 4th day	Sat-16/05	14:00				Cell density measurement (Charlie).
5	Cell densities 7th day	Tue-19/05	14:00				Cell density measurement (Delta).

Table B.1.3 – 2 - Protocol

Results

Figure B.1.3 – 1 shows cell expansion normalised with respect to the first day. The normalisation was done respect to the first day, and not to the original seeding densities, because seeding cell density was not measured (was only calculated during sample preparation.)

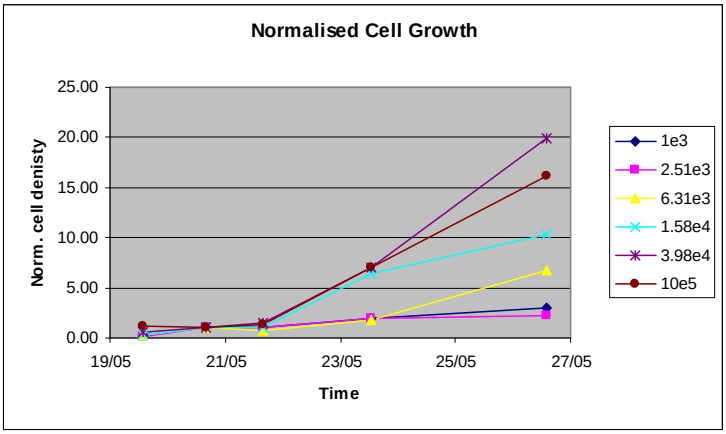


Figure B.1.3 – 1 – Normalised Cell Growth

Table B.1.3 – 3 and Figure B.1.3 – 2 show cell expansion in relation to seeding density, for the seventh day of culture.

Seeding density (cells/ml)	Fold after 7 days of culture	SD
2.5E+03	2.25	0.00
6.3E+03	6.71	1.24
1.6E+04	10.36	1.46
4.0E+04	19.86	2.89
1.0E+05	16.12	2.88

Table B.1.3 – 3 – Seeding density effect in cell growth

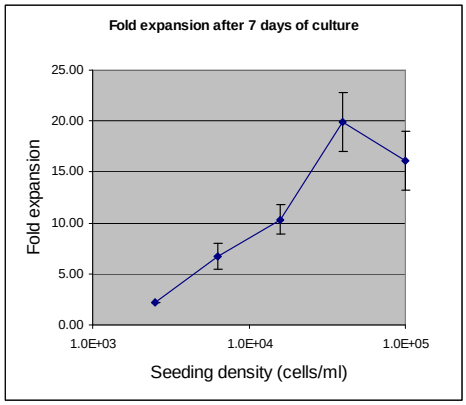


Figure B.1.3 – 2 – Seeding density effect in cell growth

Discussion and Conclusions

Figure B.1.3 - 1 shows the fold-expansion (output/input cell number) of KG1a cells related to seeding density. This result presumably is due to the lack of autocrine growing factors for low concentration of cells. The smaller fold expansion for seeding densities greater than $1.0x10^5$ is due to very high cell concentration (>$1.0x10^6$ after seven days of culture.) This effect is known for KG1a cells cultured in tissue cultured flasks (22).

Because cell growth depends on seeding density, initial biomass inoculated into the hollow fibre bioreactor dictates initial media volume and flow rates for both the intra-capillary and the extra-capillary circuits.

B.2. Cells And System

B.2.1 Hollow Fibre Bioreactor Expansion

See Chapter 8 of document for a complete description of the experiment.

B.3. System

B.3.1 Pump

The peristaltic pump is used in the hollow fibre bioreactor for extra-capillary media perfusion. The flow rates at which media is perfused vary the mass transfer characteristics of the system, a variable that has to be carefully controlled.

Aims

Determine the pump flow rate for the indicated dial position.

Material and Methods

Water was pumped from one water reservoir to another during a known amount of time. The final volume was measured and the flow-rate determined. The experiment was done for two types of tube with different diameters.

Results

Table B.3.1 – 1 and Figure B.3.1 – 1 show the relationship between pump dial position and flow rate.

Date:	23/06/97	
Pump dial	**Flow rate silicon (black-brown) (ml/min)**	**Flow rate Yellow tubing (ml/min)**
	b-b	y
1	0.18	1.13
1.5	0.62	
2	0.97	5.10
3	1.79	10.80
4		15.18
5	3.30	19.27

Table B.3.1 – 1 – Flow rate vs. dial position

A linear approximation for flow rate as a function of dial position was calculated for both type of tubing. The equation and graphical representation are given in Figure B.3.1 – 1.

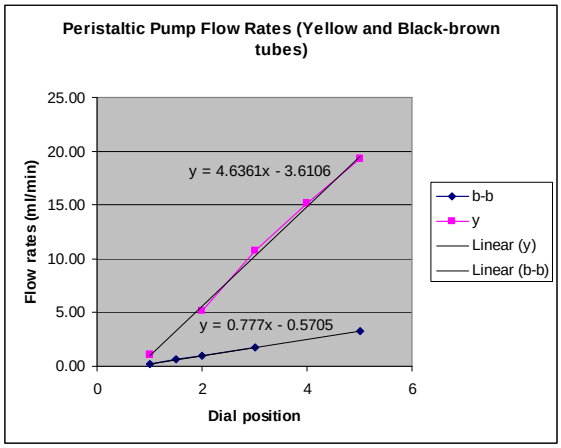


Figure B.3.1 – 1 – Flow rate vs. dial position

Discussion and Conclusions

Flow rate depends on dial position and type of tube used. The relationship between flow rate and dial position is almost linear.

For the use of the peristaltic pump in the hollow fibre bioreactor, the flow rate is determined and then the peristaltic tube and dial position defined.

A precise control of extra-capillary media flow rate will be possible using the electronic remote control of the peristaltic pump, which was not studied in this experiment.

B.3.2 Silastic Tube - Heat Transfer

(Note: the structure of this experiment is identical to the next one: B.3.3 Silastic Tube – O_2 Transfer)

Media, in the hollow fibre bioreactor, has to be at 37ºC when entering the hollow fibre module. Silastic tube is one of the elements used in the GHE module for media heating, therefore the heat transfer in silastic tube is an important parameter in the bioreactor design.

Aims

Determine maximum flow rate at which media is heated from room temperature to 37ºC for a specified length of silastic tube.

Material and Methods

Water is pumped through 50 cm of silastic tube (ID=0.040in, OD=0.085). Air convection is forced around the silastic tube. The initial and final water temperature are measured so as the surrounding convected air temperature. Measurements are taken for different water flow rates. Figure B.3.2 – 1 shows the experiment setup.

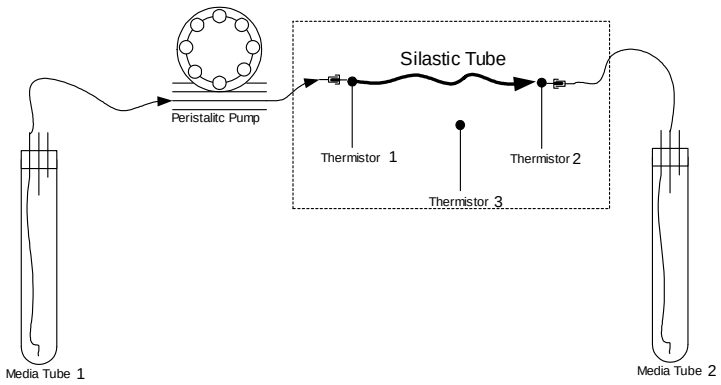


Figure B.3.2 – 1 – Heat transfer setup.

Results

Figure B.3.2 – 2 shows the output temperature of water versus 1/Flow rate. From the data, the time that the fluid has to be in the tube can be calculated.

From Figure B.3.2 – 2 a plateau is reached at 1 min/ml (P) for 50 cm of tubing (L_0).

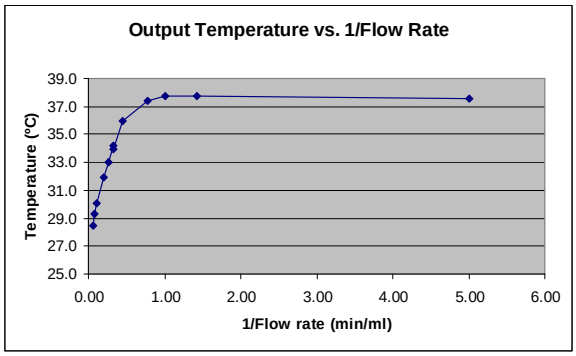


Figure B.3.2 – 2 –

The length of tube (L) required for water temperature to reach a plateau at any flow rate (F) is given in Eq. B.3.2 – 1.

$$L = F\,P\,L_0 \qquad \textbf{Eq. B.3.2 – 1}$$

Discussion and Conclusions

Heat transfer characteristics of silastic tube make it appropriate for its use in the GHE module of the bioreactor. With increase in flow rate, the length of tube has to be extended in a directly proportional fashion.

If a more complex heat transfer process has to be analyse in silastic tube (not simple equilibration with environment) a computer simulation of heat transfer can be done.

B.3.3 Silastic Tube – O_2 Transfer

(Note: the structure of this experiment is identical to the previous one: B.3.2 Silastic Tube – Heat Transfer)

O_2 partial pressure on media, in the hollow fibre bioreactor, has to be at 20.9 mmHg (atmospheric partial pressure) when entering the hollow fibre module. Silastic tube is the elements used in the GHE module for media oxygenation, therefore the O_2 transfer in silastic tube is an important parameter in the bioreactor design.

Aims

Determine maximum flow rate at which media is oxygenated from low O_2 partial pressures (<5 mmHg) up to 20.9 mmHg (atmospheric) for a specified length of silastic tube.

Material and Methods

Deoxygenated water is pumped through 50 cm of silastic tube (ID=0.040in, OD=0.085). The initial and final water O_2 concentrations are measured for different flow rates. Convection of air at 37ºC is forced around the silastic tube. Figure B.3.3 – 1 shows the experiment setup.

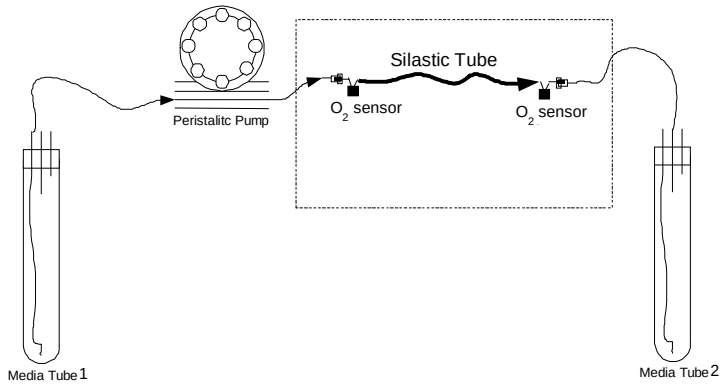


Figure B.3.3 – 1 – O_2 transfer setup.

Results

Figure B.3.3 – 2 shows the output O_2 concentration in water versus 1/Flow rate. From the data, the time that the fluid has to be in the tube can be calculated.

From Figure B.3.2 – 2 a plateau is reached at 1.5 min/ml (P) for 50 cm of tubing (L_0).

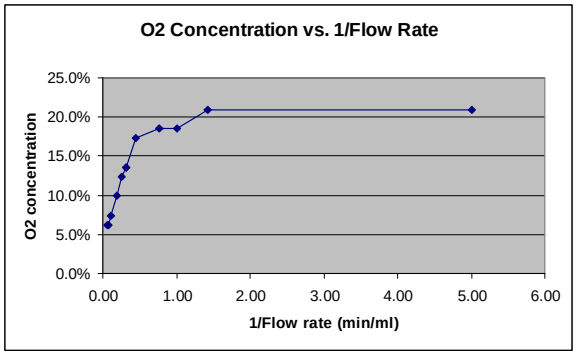


Figure B.3.3 – 2 –

The length of tube (L) required for water temperature to reach a plateau at any flow rate (F) is given in Eq. B.3.3 – 1.

$$L = F\,P\,L_0 \qquad \textbf{Eq. B.3.3 – 1}$$

Discussion and Conclusions

O_2 transfer characteristics of silastic tube make it appropriate for its use in the GHE module of the bioreactor. With increase in flow rate, the length of tube has to be extend in direct proportion to the flow rate.

If a more complex heat O_2 process has to be analyse in silastic tube (not simple equilibration atmospheric O_2 partial pressure) a computer simulation of mass transfer can be done.

B.3.4 Temperature Control

Temperature is a very important homeostatic parameter that has to be carefully controlled in the hollow fibre bioreactor. For the design of the temperature control loop and for its validation, temperature has to be constantly monitored. In this experiment the transient temperature of the bioreactor is logged

Aims

Record temperature of the hollow fibre bioreactor system during a specific period of time (up to 10 hours).

Material and Methods

The temperature control software developed includes the logging feature. Figure B.3.4 – 1 shows the front panel of the "Temp Controller.vi". To log the temperature for any period of time the "Save HISTORI DATA" button is pressed and the temperature values of the graph are saved in a text data file at one second intervals. This is all done, while the close-loop temperature control system is running For details in the temperature control system refer to Chapter 6.

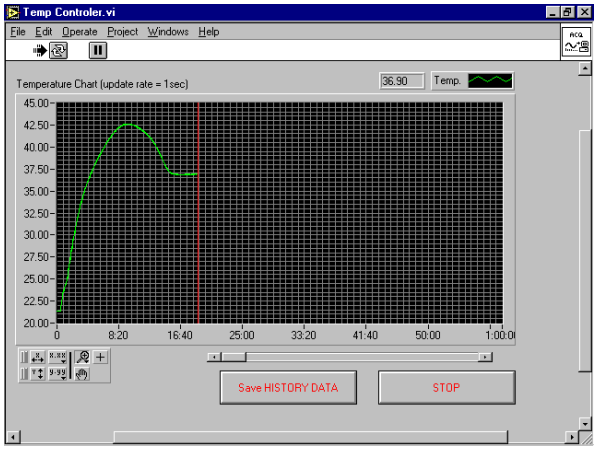


Figure B.3.4 – 1 – Temp Controller front panel.

Results

The temperature stabilises at 37 ºC and the initial overshoot follows the simulated response (Figure 18, Chapter 6) The final temperature was maintained at 37 ± 0.2 ºC

despite room temperature changes. Figure B.3.4 – 2 shows the transient temperature of the system. Figure B.3.4 – 2 was created in MATLAB from the temperature data file saved by "Temp Controller.vi"

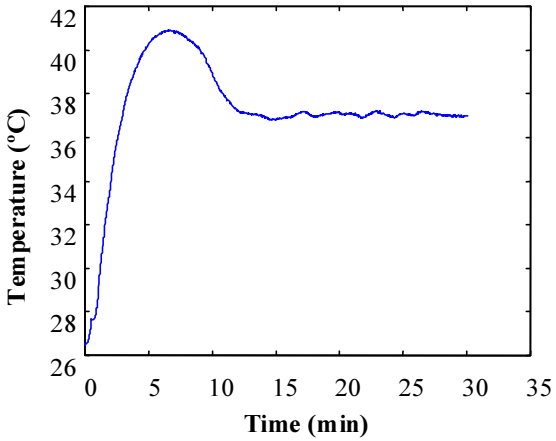


Figure B.3.4 – 2 – System temperature response

Discussion and Conclusions

Although a very simple experiment (now that the software is developed), it contains very important data for the design of the hollow fibre bioreactor. The temperature control unit model was tested and validated using the logged data. An initial over-shoot, that can be deleterious for cells inside the hollow fibre module, occurs in the initial transient. Future developments of the temperature control have to avoid over-heating (>40ºC) at any time.

Appendix C - System Parts

C.1. General View

Figure C.1 – 1 and Photo C.1 – 1 show the final system developed including labels of the parts used. All the parts and sub-parts are referenced in Table C.1 – 1

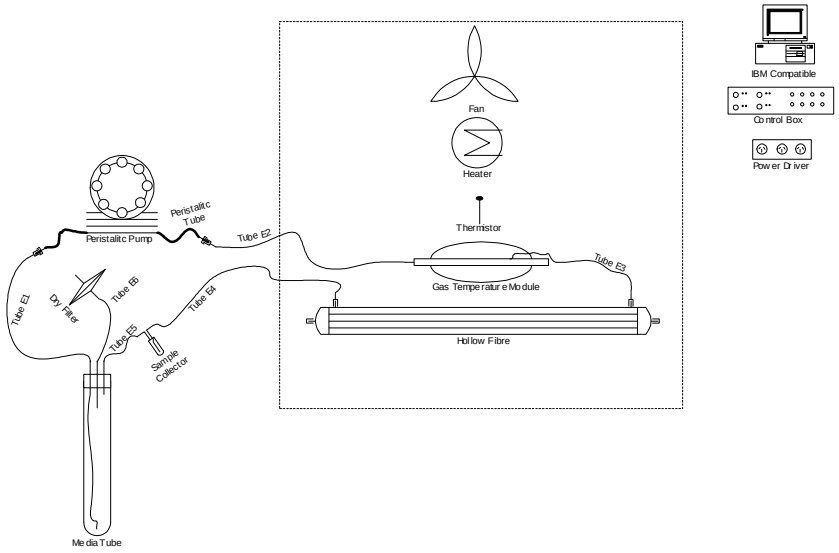
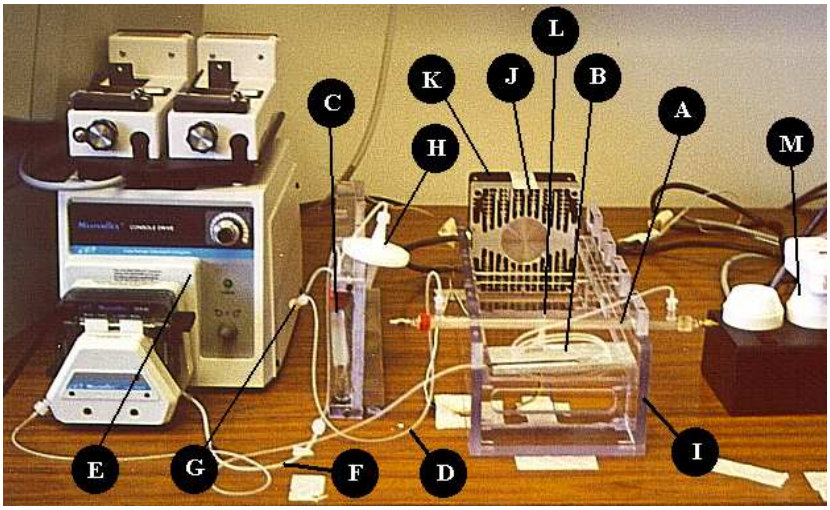


Figure C.1 - 1 - Final System



A - Hollow fibre module	H - Dry filter
B - Gas-Heat Exchange module	I - Sterilisable base
C - Media tube	J - Heater
D - Tubing	K - Fan
E - Peristaltic pump	L - Thermistor
F - Peristalitc tube	M - Power dirver
G - Sample collection unit	

Photo C.1. – 1 - Final System

Part	In-house Manuf.	# Sub-part	Sub-parts	Reference
Hollow-Fibre Module		1		See Hollow Fibre Sheet.
Gas-Heat-Exchange Module (See Gas Heat Exchange Module Sheet)	X	2	Aluminum structure	See Gas-Heat Exchange Module Sheet.
		3	Silicon Tube	SILASTIC Laboratory Tubing Cat. No. 508-005 0.040 in ID, 0.085 in. OD by Dow Corning (APS Ajax specialtychem)
		4	Metalic Tube	Graded 313 Stainless Steel tube 1.6mm OD
		5	Heat conducting Polymer	Plastic steel liquid, RS 691-246
Media Tube (see Media Tube sheet)	X	6	Glass Tube with Stopper	VACUTAINER 36 6430 (Becton Dickenson)
		7	Metalic Tubing	Syringe needle 18_G
		8	Silicon Tube	see 3
Tubing (E1, E2, E3, E4, E5, E6)		9		see 3
Peristaltic Pump		10	Precision Standard Drives with 10-Turn Seed Control and Remote Capabilites….	{Page. 832 Cole-Parmer 97-98 (Extech equipment Pty. Ltd)}
		11	L/S Multi-Channel Cartridge Pump	{Head Page. 818 Cole-Parmer 97-98 (Extech equipment Pty. Ltd)}
Peristaltic Tube		12		Silicon Part No. 116-0581-P15 REV M Color-Black/Brown Technicon Instruments Corporation SMC Pump Tubes
Fittings		13	Male luer with locking nut (E-30504-00)	Page. 316 Cole-Parmer 97-98 (Extech equipment Pty. Ltd)
		14	Male luer plug with locking nut (E-30504-20)	
		15	Female luer (E-06359-27)	
		16	Female luer plug (E-31507-53)	Page. 317 Cole-Parmer 97-98 (Extech equipment Pty. Ltd)
Sample Collection Unit (see Sampling Collection Unit sheet)	X	17	Plastic connector	see 16
		18	Metalic tube	see 7
		19	Injection site	Cat. No. 50-251 Injection Site Male luer slip TUTA Laboratories Pty. Ltd
Dry Filter		20		Millex - FG50 0.2micm Filter (MILLPORE)
Sterilisable base	X	21		See Sterilisable base sheet

(Continue next page)

Base with heating and air circulation elements	X	22	Base	
		23	Heater	RS 224-559
		24	Fan	RS 498-081
Temperature sensor		25	Thermistor	RS 151-221
Cover	X	26		See Cover sheet
Control Box	X	27	Temperature Circuit	See Control Box sheet
Power driver	X	28	OptoTRIAC	MOC3021; RS 195-4116
		29	Diode	1N4148
		30	Resistor	1Kohm,1/4 watt; RS 135-847
		31	Fuse	10 AMP
		32	TRIAC	BT137-500
Computer		33	Hardware	PC 486
		34	Software	LabView - National Instruments
		35	DAQ Board	LAB-PC National Instruments

Table C.1. – 1 - Parts and Sub-parts reference table

C.2. Hollow Fibre Module

Figure C.2 – 1 shows a sketch of the hollow fibre module and Table C.2 – 1 shows the characteristics that identify the type of module used.

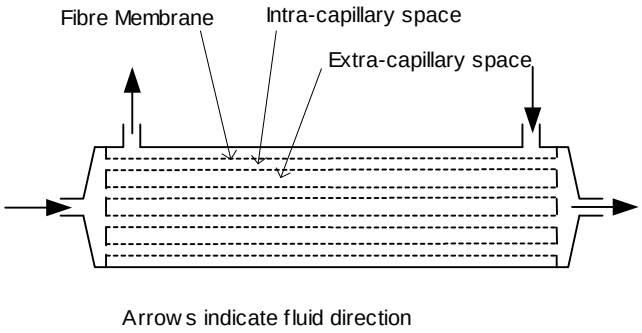


Figure C.2 – 1 – Hollow fibre module sketch

Manufacturer:	**Membrantechnikum**

Characteristic	**#**	**unit**
MiniTyp:	Minimodul 6mm	
Typ:	Cuprophane F1	
Polymer	Cellulose	
Form:	Kapillarmembran	
F-Nr	0662	
Wand	8	micm
Lumen	200	micm
Fadenzahl	174	
Gesamtflaeche	0.0272	m2
Gesamtlaenge	23	cm
Effective Flaeche	0.0248	m2
Effective Laenge	21	cm

Table C.2 – 1 – Hollow fibre module characteristics

C.3. Gas-Heat Exchange (GHE) Module

Figure C.3 – 1 shows the design of the aluminium base of the GHE module.

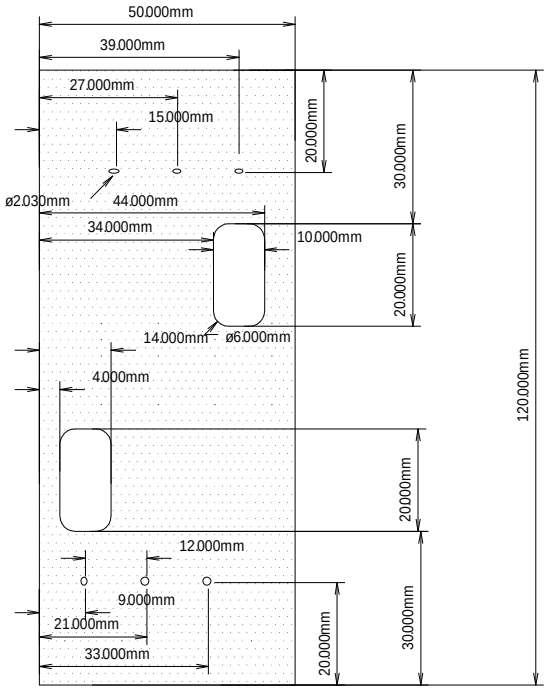


Figure C-3 - 1 - Aluminium Base

Figure C-3 - 2 shows how the GHE module is assembled. The groove in where the metallic tube fits is filled with "Plastic Steel".

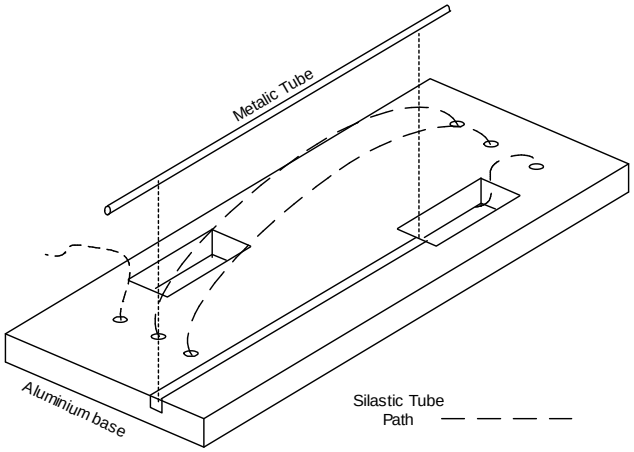


Figure C-3 - GHE module assembly

C.4. Media Tube & Dry Filter

Figure C-4 - 1 shows the Media Tube and the Dry Filter attached to it. The tube and stopper are available as a commercial unit, for blood sampling. The metallic tubes used for the input, output and connection to the Dry Filter, for ventilation, are 18G syringe needles cut. A silastic tube is connected to the output needle, inside the tube, to extend it all the way down to the bottom.

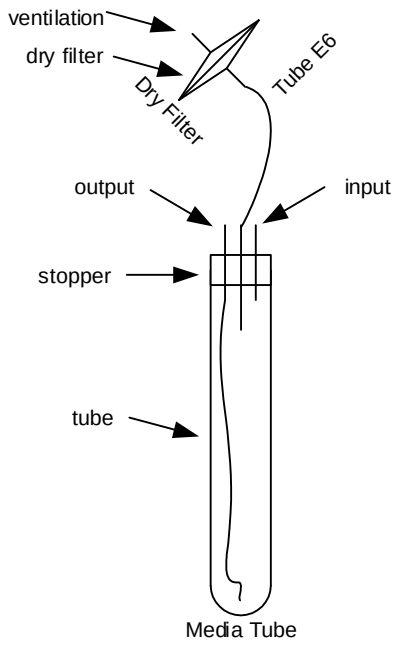


Figure C-4 – 1 - Media Tube and Dry Filter

C.5. Sample Collection Unit

The Sample Collection Unit was built with a female luer tube connector (see 16), a syringe needle 18G (see 7) and Injection Site (see 21). The Injection Site (male luer) fits into the female luer tube connector, which is the output of the Sample Collection Unit. The syringe needle, the input, passes through the plastic of the female luer tube connector.

Figure C-5 - 1 shows the diagrammatic representation of the Sample Collection Unit.

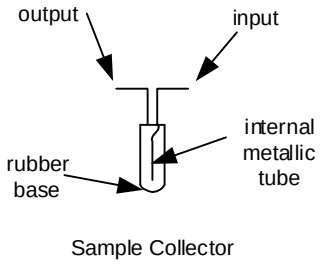


Figure C-5 - Sample Collection Unit

Figure C-5 - 2 shows a detailed diagram of the Sample Collection Unit, its parts and flow path.

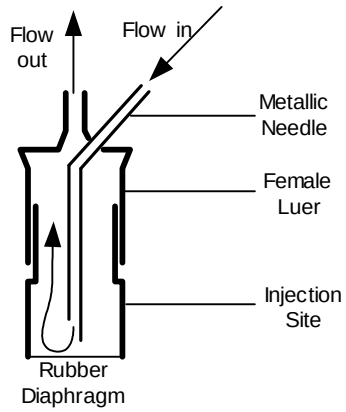


Figure C-5 - 2- Sample Collection Unit parts and flow path

C.6. Sterilisable Base

Figures C.6 – 1 and C.6 – 2 show the design of the two components of the sterilisable base, implemented in polycarbonate. The Media Tube Holder was used to support the media tube, and the portable Hollow Fibre Base was used to support the hollow fibre module and GHE module, as shown in Photo C-1.

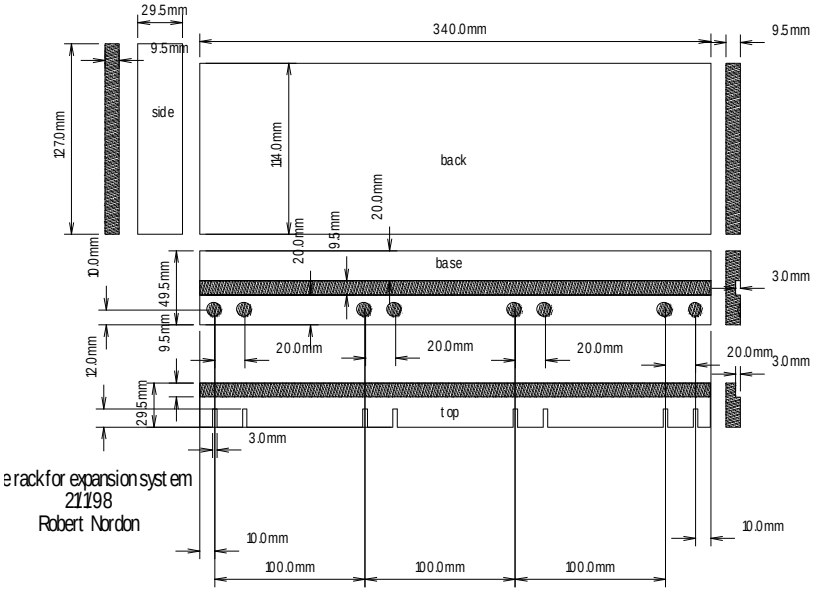


Figure C.6 – 1 – Sterilisable Media Tube Holder

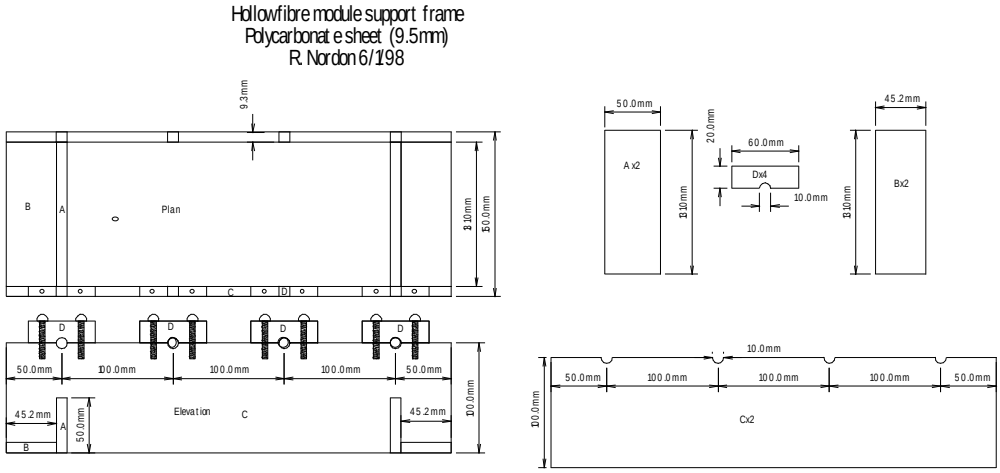


Figure C.6 – 2 – Sterilisable Hollow Fibre Base

C.7. Main Base

As shown in Photo C-1 the system was mounted in a laboratory bench. Fan and heater were attached to the bench and the precise position of the portable sterilisable base (C.6) was clearly marked to ensure proper air convection.

C.8. Perspex Lid

Figure C.8 – 1 shows the perspex lid constructed to cover the temperature control space.

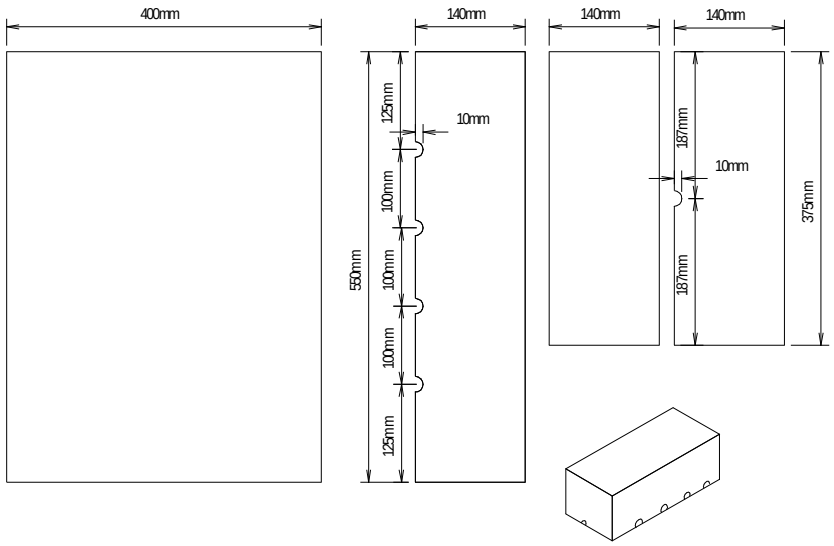


Figure C.8 – Perspex Lid Diagram

C.9. Temperature Circuit

Figure C.9 – 1 shows the circuit configuration used for excitation of the thermistors. Resistors R1, R2, and R3 are standard ¼ watt resistors. Calibration of the temperature system was done in software, same as thermistor linearisation.

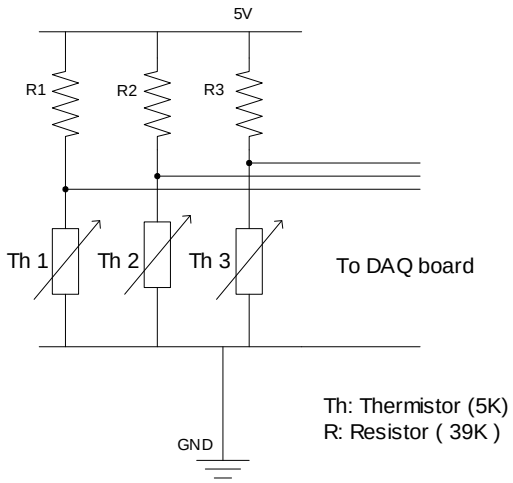


Figure C.9 – 1 – Temperature circuit

C.10. Power Driver

Figure the circuit diagram of the power driver developed for temperature control. The maximum current in the power circuit is (10A) is defined by the TRIAC. The power circuit is opto-isolated (MOC3021).

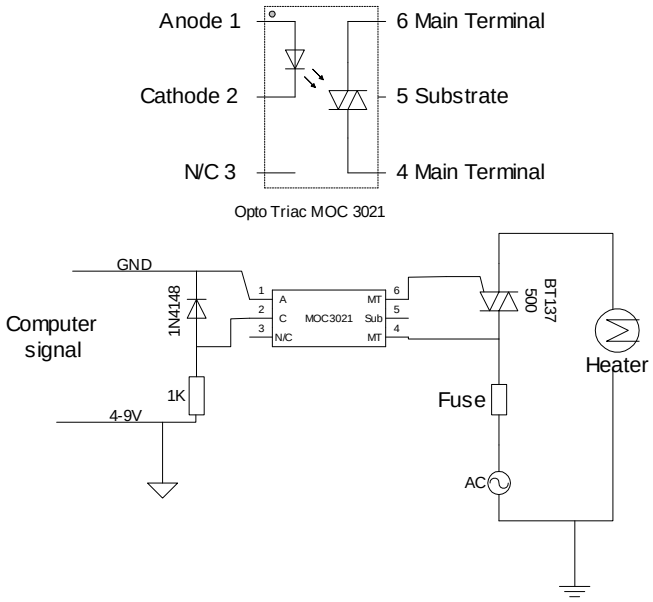


Figure C.10 – 1 – Power driver circuit

Appendix D - System Software

The software was developed in LabVIEW. For the temperature control system to work; both, “Temp Controller.vi” and “Heater Control.vi” have to be running at the same time. Figure D-1 shows the front panel of the “Temp Controller.vi” and Figure D-2 shows its diagram.

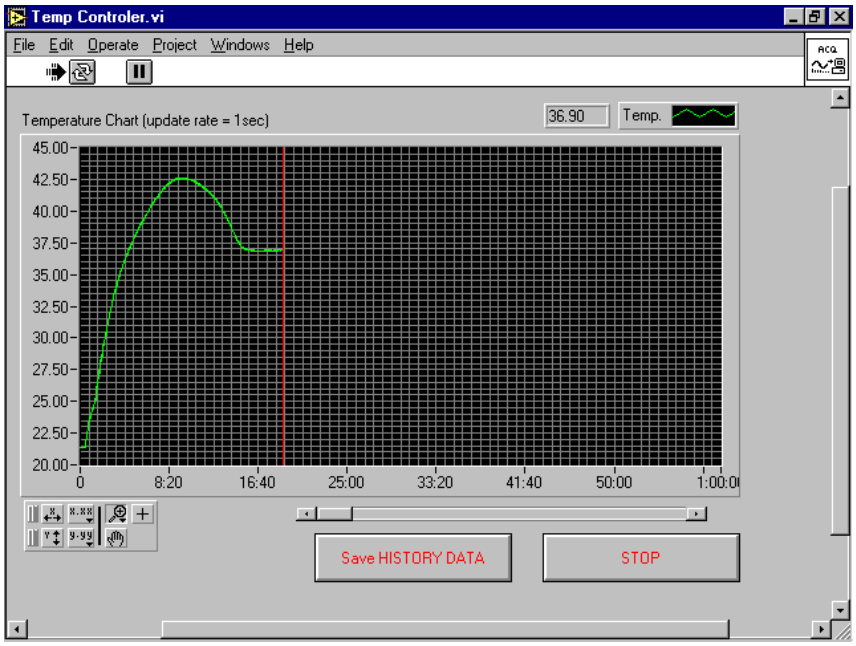


Figure D-1 – Temp Controller Front Panel

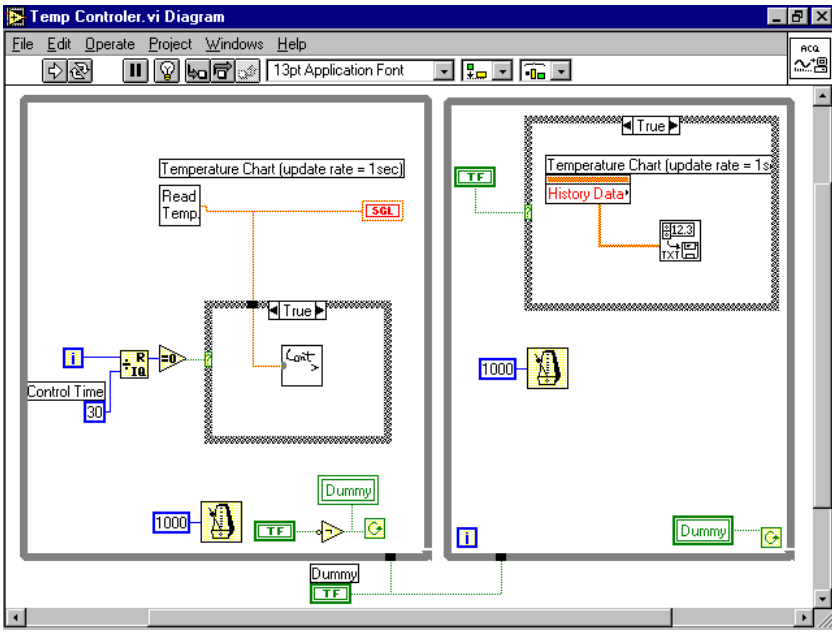


Figure D-2 – Temp Controller Diagram

The front panel of “Heater Control.vi” is shown in figures D-3. The “Heater Control.vi” was developed based in the “Function Generator.vi” which is part of the LabVIEW examples.

Figures D-1 to D-3 are examples of the VIs developed in this project. All the VIs are in the attached CD and documentation is include in the same VI.

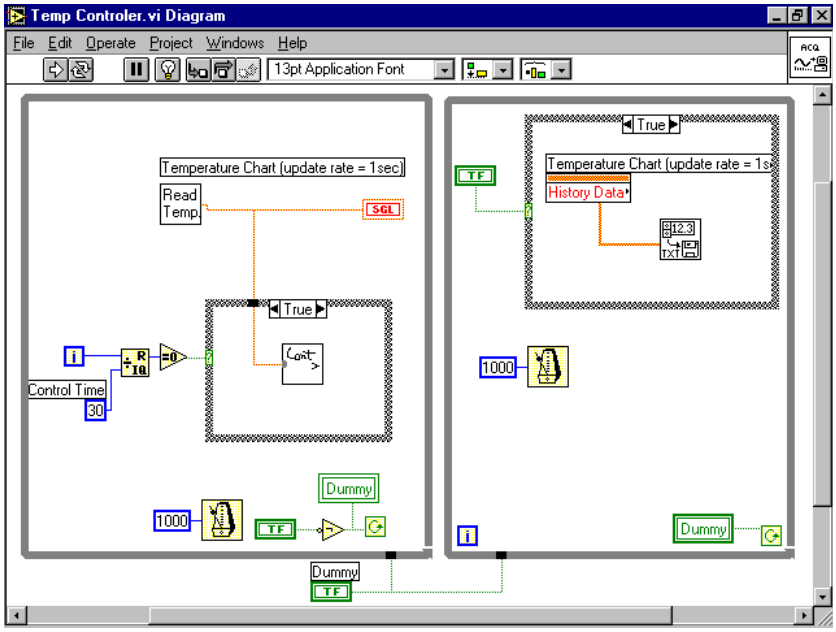


Figure D-3 – Temp Controller Diagram

Figure D-4 shows the hierarchy of VIs developed and used for the control system. Because the VIs include their own documentation and because of the graphical nature of LabVIEW the appropriate way to understand the system software is to refer it directly in the computer.

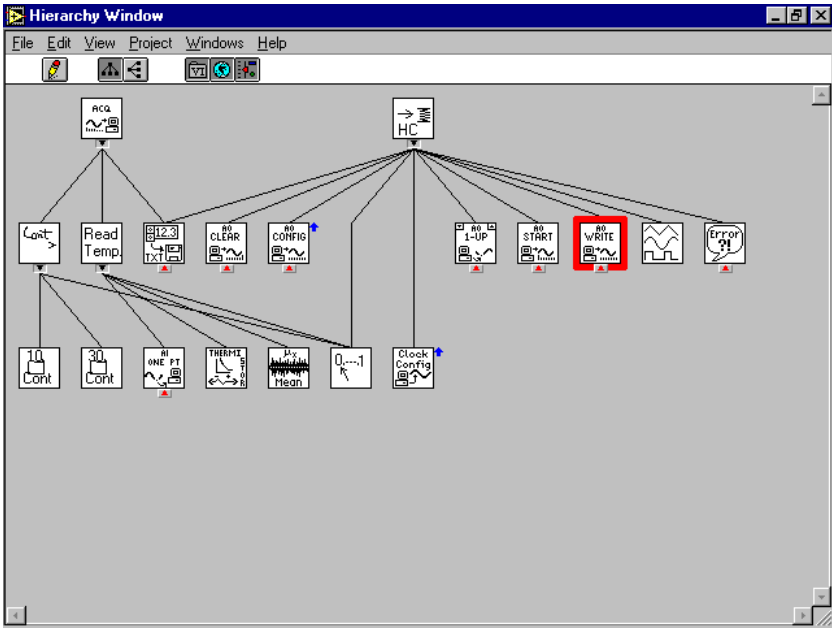


Figure D-3 – VI herarchy

Appendix E - Computational Tools

E.1. Cell Counting Software

This software was developed in "WIT" (`...\Project\Appendices\Tools\Cell countig software\Image Analysis\...`), to count cells in the lumen of a hollow fibre. The principal program, is shown in Diagram E.1 – 1. This program allows the user to select a region of the visualised area, where the analysis (counting) is done. A set of parameters is given to the sub-program "Cellsel.igr" (Diagram E.1 – 2) to define the selection characteristics of a cell. In the analysed area all the "blobs" (refer WIT definition) that satisfy the criteria are counted as cells. A copy of the software is provided with the attached CD.

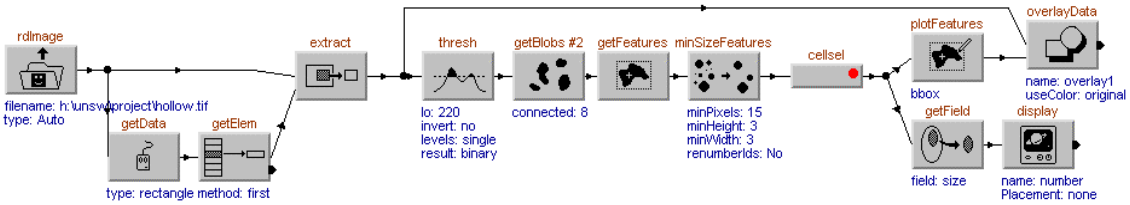


Diagram E.1 – 1 – "Separate.igr" (Wit diagram)

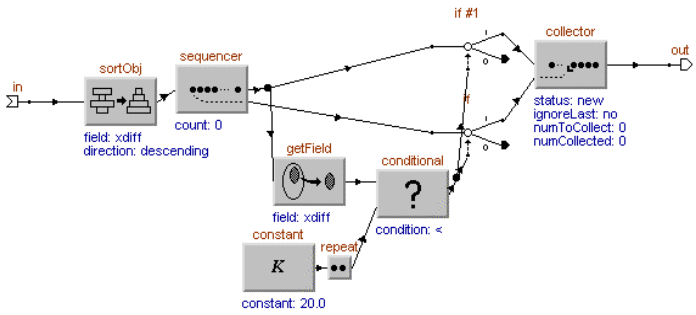


Diagram E.1 – 2 "Cellsel.igr" (Wit diagram)

E.2. Cell Handling

A set of tools was developed in EXCEL (`...\Project\Appendices\Tools\Cell Handling\Tools.xls`) to help with calculations related with cell manipulation such as dilution, expansion and others. All these tools are included in the attached CD.

E.2.1 Dimensions

Used in calculating relationships between hollow fibre dimensions and cell dimensions. An example of the "Dimensions" EXCEL sheet is presented as follows.

SEEDING	
density (cell/ml):	**3.28E+07**
HOLLOW FIBER MODULE	
N:	**2.00E+02**
lenght (m):	**2.00E-01**
diameter(m):	2.00E-04
surface area(m2):	2.51E-02
internal volume(m3):	1.26E-06
depth (m3/m2):	0.00005
# cell to fill:	4.12E+07
CELL	
diam (m):	8.00E-06
surface area (m2):	3.77E-11
real volume (m3):	5.03E-17
used volume (m3):	1.00E-15
used area (m2):	1.00E-10
CELL - MODULE	
Cell pack:	1.26E+09
Packing density(cell/ml):	1.00E+09
% of packing:	3%
Density for a line (cell/ml):	3.18E+06
FLUSK	
volume (m3):	**2.00E-05**
cell density (#cell/ml):	**1.50E+06**
total # cells:	3.00E+07
MODULE - FLUSK	
density M/F:	
# cells M/F:	

Example E.2.1 - Dimensions

E.2.2 Cell Consumption

Used to calculate relationships between cell consumption and cell density. An example of the “Cell Consumption” EXCEL sheet is presented as follows.

SEEDING
density (cell/ml): **2.51E+05** Specific glucose uptake (mmol/1e6cells/day): **6**

x2 Time (h): **3.00E+01**

K (1/h): 2.31E-02
Tao (h): 4.33E+01

	SAME CONSUMPTION TIMES				DELTA T=constant		Log average Cell density (cells/ml):	Glucose Uptake (mmol):
	# cells:	folds:	dT (h):	Time (h):	#cells:	Time(h):		
	2.51E+05			0.00	2.51E+05	0.00		
	1.56E+06	6.20E+00	**79.00**	79.00	2.57E+05	**1.00**	7.16E+05	1.41E+01
	2.86E+06	1.84E+00	26.36	105.36	2.63E+05	2.00	2.14E+06	2.83E+01
	4.17E+06	1.46E+00	16.27	121.63	2.69E+05	3.00	3.48E+06	4.24E+01
	5.48E+06	1.31E+00	11.79	133.42	2.75E+05	4.00	4.79E+06	5.65E+01
	6.78E+06	1.24E+00	9.26	142.68	2.82E+05	5.00	6.11E+06	7.07E+01
	8.09E+06	1.19E+00	7.62	150.31	2.88E+05	6.00	7.42E+06	8.48E+01
	9.40E+06	1.16E+00	6.48	156.79	2.95E+05	7.00	8.73E+06	9.89E+01
	1.07E+07	1.14E+00	5.63	162.42	3.02E+05	8.00	1.00E+07	1.13E+02
	1.20E+07	1.12E+00	4.98	167.41	3.09E+05	9.00	1.13E+07	1.27E+02
	1.33E+07	1.11E+00	4.47	171.87	3.16E+05	10.00	1.27E+07	1.41E+02
	1.46E+07	1.10E+00	4.05	175.93	3.24E+05	11.00	1.40E+07	1.55E+02
	CONSUMPTION:		Time	Folds				
New seed (# cell/ml):	**1.00E+07**	3.98E+01	159.4851	7.462781734				
Density after dT (#cell/ml):	1.13E+07	dT (h):	5.31E+00				1.06E+07	

Time(days)
6.645211

Example E.2.2 – Cell Consumption

E.2.3 Cell Dilution

Used to calculate dilution of cell in suspension. For a minimum and maximum densities, and a specified number of divisions, the intermediate cell densities are calculated as well as the media and cell requirements. An example of the "Cell Dilution" EXCEL sheet is presented as follows.

N	**6**	
Max density:	**1.00E+07**	
Min density:	**5.00E+05**	
# of samples:	**6**	
Vol. sample (ml):	**7.00E-01**	
Resolution:	**0.1**	
		Total:
# Cells:	1.60E+07	9.60E+07
Media (ml):	4.64	27.82

Dilution: 1.8206

	Dens. (#cell/ml):	Vol S. (ml):	Dilution:	V S-1 (ml):	V media (ml):	V needed (ml):	Left (ml):	# Cells:	Max Volume (ml):
1	**1.00E+07**	**0.7**			1.60	1.60		1.60E+07	1.60
2	**5.49E+06**	**0.7**	1.82	0.90	0.74	1.60	0.04		1.64
3	**3.02E+06**	**0.7**	1.82	0.90	0.74	1.50	0.14		1.64
4	**1.66E+06**	**0.7**	1.82	0.80	0.66	1.40	0.06		1.46
5	**9.10E+05**	**0.7**	1.82	0.70	0.57	1.10	0.17		1.27
6	**5.00E+05**	**0.7**	1.82	0.40	0.33	0.70	0.03		0.73
7	**2.75E+05**	**0**	1.82	0.00	0.00	0.00	0.00		0.00
8	**1.51E+05**	**0**	1.82	0.00	0.00	0.00	0.00		0.00
9	**8.29E+04**	**0**	1.82	0.00	0.00	0.00	0.00		0.00
10	**4.55E+04**	**0**	1.82	0.00	0.00	0.00	0.00		0.00
11	**2.50E+04**	**0**	1.82	0.00	0.00	0.00	0.00		0.00
12	**1.37E+04**	**0**	1.82	0.00	0.00	0.00	0.00		0.00

Example E.2.3 – Cell Dilution

E.2.4 Time

Used to calculate the time intervals between events in an experiment. An example of the "Time" EXCEL sheet is presented as follows.

Time

Starting point (time):	**23/12/97 8:00**	Doubling time (h):	**30:00:00**
Number of samples:	**12**	Expansion (x),(2^x):	3.17 8.98
Experiment lenth:	3 days, 23:00 h:min 92:00:00		

	Δt		
1		Tue-23/12	8:00
2	3:00	Tue-23/12	11:00
3	3:00	Tue-23/12	14:00
4	3:00	Tue-23/12	17:00
5	6:00	Tue-23/12	23:00
6	8:00	Wed-24/12	7:00
7	12:00	Wed-24/12	19:00
8	12:00	Thu-25/12	7:00
9	12:00	Thu-25/12	19:00
10	12:00	Fri-26/12	7:00
11	12:00	Fri-26/12	19:00
12	12:00	Sat-27/12	7:00

Example E.2.4 - Time

E.3. HF Mass Trans - Algebraic

This EXCEL sheet, included in the attached CD (`...\Project\Appendices\HF Mass Trans Algebraic\MassTransfer.xls`), helps to calculate the mass transfer of a substance in a cylindrical geometry using the Sherwood number approximation. An example of the "HF Mass Trans" EXCEL sheet is presented as follows.

Mass Transfer For Hollow Fibre Modules

Module:	
Membrane Type:	**Cuprophane**
Number of fibres:	**174**
Fibre internal diameter, wet (μm):	**270**
Total intra-capillary area (cm^2):	9.96E-02
Membrane wall thickness, wet (μm):	**8**
Fibre length (cm):	**20**
Surface Area (cm^2):	2.95E+02
Internal shell diameter (mm):	**27**
Fluid external free area (cm^2):	5.61E+00
Total radius for one fibre (μm):	723.68

Fluxes:	
Current type:	**Counter-current**
Qb (ml/min):	**120**
Velintra (cm/min):	1.20E+03
Δp (mmHg):	
Qd (ml/min):	**120**
Velextra (cm/min):	2.14E+01
Δp (mmHg):	

Solute:	
Solute name:	**B12**
MW:	**1355**
1 / Expected Cuprophane permeability, form Fig class (31/7/97) (min/cm):	***333.3***
Real Cuprophane resistance (min/cm):	**20.8**
Expected aqueous diffusion coefficient, form Fig class (31/7/97) (cm^2/sec x 10^-5):	***0.22***
Real aqueous diffusion Db (cm^2/sec x 10^-5):	**2.80**

Analysis:	
X (dimensionles length):	0.038
Sh (Sherwood Number):	5.519679873
k (cm/min);	0.343
Rintra (min/cm):	2.91
Rt cupeoph (min/cm) {1/k0}:	23.74
Rt m. cuproph	88%
k0A cuproph (cm^3/min):	12.43

Figure E.3 – 1 Hollow fibre mass transfer example

E.4. HF Mass Trans - Computational Model

Computational Solution For Concentration In a Cylindrical-Geometry

The best way to understand the function of all the files and subroutines that conform the simulator, is to go directly to the code, following the logical structure and comments.

E.4.1 Structure

This computational model was developed in MATLAB. The main program call sub-programs and functions also developed in MATLAB

E.4.2 How To Use The Simulator

Running the simulation

Type "HollowFibreModel" in the main MATLAB window to call the main command file (after the path of the sub-programs and functions is included in the search paths.)

Tailoring the simulator

The user can do the following changes to tailor the simulator for his/her application.

1. To change simulation constants (which define the simulated system) the file "ConstatDeclaration.m" has to be modified.
2. To adjust simulation parameters the file "SimulationParameters" has to be modified.
3. The files "CalculateIntra.m" and "CalculateExtra.m" can be modified to change the conditions for the simulation, ie boundary conditions, flow direction, etc.

E.4.3 Code

The MATLAB code is presented in the following pages.

```
% File: ...\Project\Appendices\Tools\HF Mass Trans Model
%          \Computational Model\HollowFibreModel.m
%
% This program calculates the concentration of any substance in a
% hollow fibre module, given the appropriate parameters.

ConstantDeclaration;
VariablesDeclaration;
SimulationParameters;

% Repeat for the number of iterations of NoRemeshing
for i=1:NoRemeshing

   % Resize the variables for the new mesh
   ResizeVariables;

   % Setup parameters for 'ScreenInfo.m'
   ScreenInfoSetup;

   %Interate until error conditions match
   errorc=inf;
   while errorc>errcV(i)
   % Calculate new concentrations cnewIntra and cnewExtra from cIn-
tra
   % and cExtra

      % Intra-capillary space
      CalculateIntra;

      % Extra-capillary space
      CalculateExtra;

      % Calculate cycle error
      CalculateError;

      % Screen Information
      ScreenInfo;
   end

   % plot before remeshing
   ok=replot(cExtra, cIntra, cerrExtra, cerrIntra);
end

'Finish!!!'
```

```
% File: ...\Project\Appendices\Tools\HF Mass Trans Model
%          \Computational Model\SubFiles\ConstantDeclaration.m
%
% ****              ConstantDeclarations            ****

% *********************   Solute   *********************
% Diffusion coefficient of membrane (cm^2/sec)

%      Permeability of the MEMBRANE (cm/sec) (Class notes Ross)
PMem   = (8.0e-4); %100/(2*pi*rI*4);
%      Diffusion constant in INTERNAL fluid (cm^2/sec)
DIntra = 2.8e-5;
%      Diffusion constant in EXTERNAL fluid (cm^2/sec)
DExtra = 2.8e-5;

% *********************   Module   *********************
%                 No Fibres
NoFibres          = 174;
%                 Internal shell diameter (cm)
ShellInternalDiam = 5.0;

% *******************   Fibre   ***********************
MemThickness = 8e-4;               %Membrane Thickness (cm)
rI           = 135e-4;             %Internal Radius  =  Diameter/2
(cm)
FibreLength  = 20;                 %Fibre length (cm)
QIntra       = (49/60)/NoFibres;      %Intracapillary flux
(cm^3/sec)
VI           = QIntra/(pi*rI^2);   %Mean fluid velocity intracapi-
llary (cm/sec)

% *************   Extra-capillary Space   ***************
%      Infinit radius (cm)
rEInf  = 200e-4;
%      Minium radius (cm)
rEMin  = rI+MemThickness;
%      Extracapillary flux (cm^3/sec)
QExtra = (45/60)/NoFibres;
%      Flow velocity extracapillary (cm/sec)
VE     = QExtra/(pi*((rEInf^2)-(rEMin^2)));
```

```
% File: ...\Project\Appendices\Tools\HF Mass Trans Model
%          \Computational Model\SubFiles\VariablesDeclaration.m
%

% ***************    Variables Declaration      ***********

% ****************    Define deltaT      ******************
deltaT = 0;

% *************    Define new mesh sizes      *************
xDiv        = 1;
rDivIntra   = 1;
rDivExtra   = 1;
deltax      = FibreLength/xDiv;
deltarIntra = rI/rDivIntra;
deltarExtra = (rEInf-rEMin)/rDivExtra;

% ***************    Intra-capillary variables      *******
cIntra                = zeros(rDivIntra,xDiv);
cnewIntra             = zeros(rDivIntra,xDiv);
NIntra                = zeros(rDivIntra,xDiv);
deltaNIntra           = zeros(rDivIntra,xDiv);
deltaNIntraAxialConv  = zeros(rDivIntra,xDiv);
deltaNIntraAxialDiff  = zeros(rDivIntra,xDiv);
deltaNIntraRadialDiff = zeros(rDivIntra,xDiv);
deltaNIntraMembrane   = zeros(1,xDiv);
rIntra                = zeros(rDivIntra,1);
vIntra                = zeros(rDivIntra,1);
VolIntra              = zeros(rDivIntra,1);
rMeanIntra            = zeros(rDivIntra,1);
SmallfIntra           = zeros(rDivIntra-1,1);
LargefIntra           = zeros(rDivIntra-1,1);

% *************    Extra-capillary variables      **********
cExtra                = zeros(rDivExtra,xDiv);
cnewExtra             = zeros(rDivExtra,xDiv);
NExtra                = zeros(rDivExtra,xDiv);
deltaNExtra           = zeros(rDivExtra,xDiv);
deltaNExtraAxialConv  = zeros(rDivExtra,xDiv);
deltaNExtraAxialDiff  = zeros(rDivExtra,xDiv);
deltaNExtraRadialDiff = zeros(rDivExtra,xDiv);
deltaNExtraMembrane   = zeros(1,xDiv);
rExtra                = zeros(rDivExtra,1);
vExtra                = zeros(rDivExtra,1);
VolExtra              = zeros(rDivExtra,1);
rMeanExtra            = zeros(rDivExtra,1);
SmallfExtra           = zeros(rDivExtra-1,1);
LargefExtra           = zeros(rDivExtra-1,1);

% *****    Intra-capillary inital concentration      *******
c0Intra = zeros(rDivIntra,1);

% ******    Extra-capillary inital concentration      ******
c0Extra = zeros(rDivExtra,1);
```

```
% File: ...\Project\Appendices\Tools\HF Mass Trans Model
%          \Computational Model\SubFiles\SimulationParameters.m
%

%**********           Simulation Parameters     ********

% number of remeshings
NoRemeshing = 1;

% mesh size for NoRemeshing steps
xDivV = [10;25;50];
rDivIntraV = [6;12;25];
rDivExtraV = [4;10;15];

% DeltaT (sec)
deltaTV = [5e-3; 1e-4; 5e-4];

%error conditions for NoRemeshing steps
errcV = [1e-8; 1e-8; 1e-10];
```

```
% File: ...\Project\Appendices\Tools\HF Mass Trans Model
%          \Computational Model\SubFiles\ResizeVariables.m
%

% *********        Resize/refresh Variables       *************

% *************           Define deltaT       *****************
deltaT = deltaTV(i);

% ***********             Define new mesh sizes     ***********
xDiv        = xDivV(i);
rDivIntra   = rDivIntraV(i);
rDivExtra   = rDivExtraV(i);
deltax      = FibreLength/xDiv;
deltarIntra = rI/rDivIntra;
deltarExtra = (rEInf-rEMin)/rDivExtra;

% ********      Intra-capillary variables   ******************
cIntra                = remesh(cIntra,rDivIntra,xDiv);
cnewIntra             = cIntra;
deltaNIntra           = zeros(rDivIntra,xDiv);
deltaNIntraAxialConv  = zeros(rDivIntra,xDiv);
deltaNIntraAxialDiff  = zeros(rDivIntra,xDiv);
deltaNIntraRadialDiff = zeros(rDivIntra,xDiv);
deltaNIntraMembrane   = zeros(1,xDiv);
rIntra                = zeros(rDivIntra,1);
vIntra                = zeros(rDivIntra,1);
VolIntra              = zeros(rDivIntra,1);
AreaIntra             = zeros(rDivIntra,1);
deltaVolIntra         = zeros(rDivIntra,1);
RadDiffIntra          = zeros(rDivIntra,1);
for n = 1:rDivIntra
   rIntra(n)        = deltarIntra*n;
   vIntra(n)        = (VI*2)*(1-(rIntra(n)-deltarIntra/3)^2/rI^2);
   AreaIntra(n)     = pi*((rIntra(n)^2)-((rIntra(n)-
deltarIntra)^2));
   VolIntra(n)      = AreaIntra(n)*deltax;
   deltaVolIntra(n) = (AreaIntra(n)*deltaT)*vIntra(n);
end
AxiDiffIntra        = AreaIntra*DIntra*deltaT/deltax;
RadDiffIntra(2:rDivIntra) =
2*pi*rIntra(2:rDivIntra)*deltax*DIntra*deltaT/deltarIntra;

% ********      Extra-capillary variables   ******************
cExtra                = remesh(cExtra,rDivExtra,xDiv);
cnewExtra             = cExtra;
deltaNExtra           = zeros(rDivExtra,xDiv);
deltaNExtraAxialConv  = zeros(rDivExtra,xDiv);
deltaNExtraAxialDiff  = zeros(rDivExtra,xDiv);
deltaNExtraRadialDiff = zeros(rDivExtra,xDiv);
deltaNExtraMembrane   = zeros(1,xDiv);
rExtra                = zeros(rDivExtra,1);
vExtra                = zeros(rDivExtra,1);
VolExtra              = zeros(rDivExtra,1);
AreaExtra             = zeros(rDivExtra,1);
deltaVolExtra         = zeros(rDivExtra,1);
RadDiffExtra          = zeros(rDivExtra,1);
for n = 1:rDivExtra
   rExtra(n)        = rEMin+deltarExtra*n;
   vExtra(n)        = VE;
```

```
   AreaExtra(n)     = pi*((rExtra(n)^2)-((rExtra(n)-
deltarExtra)^2));
   VolExtra(n)      = AreaExtra(n)*deltax;
   deltaVolExtra(n) = (AreaExtra(n)*deltaT)*vExtra(n);
end
AxiDiffExtra        = AreaExtra*DExtra*deltaT/deltax;
RadDiffExtra(2:rDivExtra) =
2*pi*rExtra(2:rDivExtra)*deltax*DExtra*deltaT/deltarExtra;

% *****    Intra-capillary inital concentration      *******
c0Intra=zeros(rDivIntra,1);
for n = 1:rDivIntra
   c0Intra(n,1) = 0;
end

% ******    Extra-capillary inital concentration      ******
c0Extra=zeros(rDivExtra,1);
for n = 1:rDivExtra
   c0Extra(n,1) = 0.05;
end
```

```
% File: ...\Project\Appendices\Tools\HF Mass Trans Model
%          \Computational Model\SubFiles\ScreenInfo.m
%

% plot after 'plotcycle' number of cycles
if plotnow==0
   ok=replot(cExtra, cIntra, cerrExtra, cerrIntra);

   % Internal bulk concentration for all the length
   for j=1:xDiv
      N=0;
      VolTot=0;
      N=sum(deltaVolIntra.*cIntra(:,j));
      VolTot=sum(deltaVolIntra);
      NIntra(j)=(N/VolTot)*QIntra*NoFibres;
   end
   figure(3)
   plot(NIntra);

   % External bulk concentration at the outlet
   N=0;
   VolTot=0;
   N=sum(deltaVolExtra.*cExtra(:,1));
   VolTot=sum(deltaVolExtra);
   NExtra=(N/VolTot)*QExtra*NoFibres

   % Remeshing cycle
   i

   %Start new backward count for next plot
   plotnow=plotcycle;
else
   % Decrease backward count for next plot
   plotnow=plotnow-1;
end
```

```
% File: ...\Project\Appendices\Tools\HF Mass Trans Model
%          \Computational Model\SubFiles\CalculateIntra.m
%

%********** deltaNIntraAxialConv **********
deltaNIntraAxialConv(:,2:xDiv) = (deltaVolIntra*ones(1,xDiv-
1)).*cIntra(:,1:xDiv-1);
deltaNIntraAxialConv(:,1) = deltaVolIntra.*c0Intra;
deltaNIntraAxialConv = deltaNIntraAxialConv - ([deltaNIntraAxial-
Conv(:,2:xDiv),deltaVolIntra.*cIntra(:,xDiv)]);

%********** deltaNIntraAxialDiff **********
deltaNIntraAxialDiff(:,2:xDiv) = (AxiDiffIntra*ones(1,xDiv-
1)).*(cIntra(:,1:xDiv-1)-cIntra(:,2:xDiv));
deltaNIntraAxialDiff(:,1) = deltaNIntraAxialDiff(:,2);
deltaNIntraAxialDiff = deltaNIntraAxialDiff - ([deltaNIntraAxial-
Diff(:,2:xDiv),deltaNIntraAxialDiff(:,xDiv)]);

%********** deltaNIntraRadialDiff **********
deltaNIntraRadialDiff(2:rDivIntra,:) = (RadDiffIn-
tra(2:rDivIntra,1)*ones(1,xDiv)).*(cIntra(1:rDivIntra-1,:)-
cIntra(2:rDivIntra,:));
deltaNIntraRadialDiff(1,:) = zeros(1,xDiv);
deltaNIntraRadialDiff = deltaNIntraRadialDiff - [deltaNIntraRadial-
Diff(2:rDivIntra,:);zeros(1,xDiv)];

%********** deltaNIntraMembrane **********
deltaNIntraMembrane =
2*pi*(rI+MemThickness/2)*deltax*PMem*deltaT*(cExtra(1,:)-
cIntra(rDivIntra,:));

%********** deltaNCellConsumption **********
deltaNCellConsumption = (VolIntra*ones(1,xDiv))*4.0e-17*deltaT*1e10;


deltaNIntra = deltaNIntraAxialConv + deltaNIntraRadialDiff + delta-
NIntraAxialDiff;% - deltaNCellConsumption
deltaNIntra(rDivIntra,:) = deltaNIntra(rDivIntra,:) + deltaNIntra-
Membrane;

cnewIntra = cIntra + deltaNIntra./(VolIntra*ones(1,xDiv));

%cnewIntra(rDivIntra,:)=ones(1,xDiv);
```

```
% File: ...\Project\Appendices\Tools\HF Mass Trans Model
%          \Computational Model\SubFiles\CalculateExtra.m
%

%********** deltaNExtraAxialConv **********
deltaNExtraAxialConv(:,1:xDiv-1) = (deltaVolExtra*ones(1,xDiv-
1)).*cExtra(:,2:xDiv);
deltaNExtraAxialConv(:,xDiv) = deltaVolExtra.*c0Extra;
deltaNExtraAxialConv = deltaNExtraAxialConv - ([deltaVolEx-
tra.*cExtra(:,1),deltaNExtraAxialConv(:,1:xDiv-1)]);

%********** deltaNExtraAxialDiff **********
deltaNExtraAxialDiff(:,2:xDiv) = (AxiDiffExtra*ones(1,xDiv-
1)).*(cExtra(:,1:xDiv-1)-cExtra(:,2:xDiv));
deltaNExtraAxialDiff(:,1) = deltaNExtraAxialDiff(:,2);
deltaNExtraAxialDiff = deltaNExtraAxialDiff - ([deltaNExtraAxial-
Diff(:,2:xDiv),deltaNExtraAxialDiff(:,xDiv)]);

%********** deltaNExtraRadialDiff **********
deltaNExtraRadialDiff(2:rDivExtra,:) = (RadDiffEx-
tra(2:rDivExtra,1)*ones(1,xDiv)).*(cExtra(1:rDivExtra-1,:)-
cExtra(2:rDivExtra,:));
deltaNExtraRadialDiff(1,:) = zeros(1,xDiv);
deltaNExtraRadialDiff = deltaNExtraRadialDiff - [deltaNExtraRadial-
Diff(2:rDivExtra,:);zeros(1,xDiv)];

%********** deltaNIntraMembrane **********
deltaNExtraMembrane = -deltaNIntraMembrane;


deltaNExtra = deltaNExtraAxialConv + deltaNExtraRadialDiff + delta-
NExtraAxialDiff;
deltaNExtra(1,:) = deltaNExtra(1,:) + deltaNExtraMembrane;

cnewExtra = cExtra + deltaNExtra./(VolExtra*ones(1,xDiv));

%cnewExtra=ones(size(cnewExtra))*1;
```

```
% File: ...\Project\Appendices\Tools\HF Mass Trans Model
%          \Computational Model\SubFiles\CalculateError.m
%
% Calculates the maximum difference between the previous and new
% concentrations for all the cells in both (Intra and Extra fibre)
% meshes.
%
% Updates cIntra and cExtra matrices.

% Calculates error
cerrIntra=cIntra-cnewIntra;
cerrExtra=cExtra-cnewExtra;
errorc=max(max(max(abs(cerrIntra))),max(max(abs(cerrExtra))));

% Updates cIntra and cExtra matrices.
cIntra=cnewIntra;
cExtra=cnewExtra;
```

```
% File: ...\Project\Appendices\Tools\HF Mass Trans Model
%          \Computational Model\SubFiles\ScreenInfo.m
%

% plot after 'plotcycle' number of cycles
if plotnow==0
   ok=replot(cExtra, cIntra, cerrExtra, cerrIntra);

   % Internal bulk concentration for all the length
   for j=1:xDiv
      N=0;
      VolTot=0;
      N=sum(deltaVolIntra.*cIntra(:,j));
      VolTot=sum(deltaVolIntra);
      NIntra(j)=(N/VolTot)*QIntra*NoFibres;
   end
   figure(3)
   plot(NIntra);

   % External bulk concentration at the outlet
   N=0;
   VolTot=0;
   N=sum(deltaVolExtra.*cExtra(:,1));
   VolTot=sum(deltaVolExtra);
   NExtra=(N/VolTot)*QExtra*NoFibres

   % Remeshing cycle
   i

   %Start new backward count for next plot
   plotnow=plotcycle;
else
   % Decrease backward count for next plot
   plotnow=plotnow-1;
end
```

```
function [newmesh]=remesh(oldmesh,x,y);
%
% File: ...\Project\Appendices\Tools\HF Mass Trans Model
%          \Computational Model\SubFiles\remesh.m
%

[xold,yold]=size(oldmesh);
newmesh=zeros(x,y);

for i=1:x
   ioldhi=ceil((xold*(i-1)+x-i)/(x-1));
   ioldlow=floor((xold*(i-1)+x-i)/(x-1));
   iloc=((xold*(i-1)+x-i)/(x-1))-floor((xold*(i-1)+x-i)/(x-1));
   for j=1:y
      joldhi=ceil((yold*(j-1)+y-j)/(y-1));
      joldlow=floor((yold*(j-1)+y-j)/(y-1));
      jloc=((yold*(j-1)+y-j)/(y-1))-floor((yold*(j-1)+y-j)/(y-1));
      newvaluelow=oldmesh(ioldlow,joldlow)+(oldmesh(ioldlow,joldhi)-
oldmesh(ioldlow,joldlow))*jloc;
      newvaluehi=oldmesh(ioldhi,joldlow)+(oldmesh(ioldhi,joldhi)-
oldmesh(ioldhi,joldlow))*jloc;
      newvalue=newvaluelow+(newvaluehi-newvaluelow)*iloc;
      newmesh(i,j)=newvalue;
   end
end
```

```
function ok=replot(cExtra, cIntra, cerrExtra, cerrIntra);
%
% File: ...\Project\Appendices\Tools\HF Mass Trans Model
%          \Computational Model\SubFiles\replot.m
%

%plot contour of Extra and Intra capillary spaces

% Plot concentrations
figure(1)
v=-0.05:0.05/16:0.05;
c=[cIntra;cExtra];
contour(c,v);

% Plot errors
figure(2)
subplot 211
mesh(cerrExtra)
subplot 212
mesh(cerrIntra)

% Force showing the plots
pause(0.1)

% Dummy return parameter
ok=1;
```

E.5. System Identification

The following function were used in the temperature model identification. This software was developed in MATLAB.

Dominant pole identification.

```
% File: ...\Project\Appendices\Tools\System Identification
%          \DomPole.m
%
% This program use the data stored in wuTemp and t, to
% find its best exponential approximation.
%
% Use funciton f.m
%

load wuTemp;
load t;
[Beta0]=[30 30 50];
Beta=nlinfit(t,wuTemp,'f',Beta0)

T=f(Beta,t);

hold off
plot(t,T)
hold on
plot(t,wuTemp,'r')

save T.txt T -ascii -double
```

```
function y=f(beta,x)

% File: ...\Project\Appendices\Tools\System Identification
%          \f.m
%
% This function is called by DomPole.m, and defines the
% "form" of the function for data fit (refer MATLAB "nlinfit").
%

y=beta(1)-(beta(2)*(1-exp((-1/beta(3))*x)));
```

System Identification ARX algorithm.

```
% File: ...\Project\Appendices\Tools\System Identification
%          \identemp.m
%
% This program is used for system identification using the ARX
% identification algorithm. This set of instructions were extracted
% from MATLAB demos (identification Toolkit).
%

utemp=dtrend(noise05s(1241:1640,1));
ytemp=dtrend(noise05s(1241:1640,2));

ztemp=[noise05s(201:1240,2) noise05s(201:1240,1)];
idplot(ztemp);
pause

ztemp2=dtrend(ztemp);

irtemp=cra(ztemp2);

thtemp = arx(ztemp2,[12 12 3]);
thtemp2 = sett(thtemp,10);
present(thtemp2);
pause

yhtemp=idsim(utemp,thtemp2);
hold off
plot(yhtemp,'b')
hold on
plot(ytemp,'g')

pause

zpthtemp = th2zp(thtemp);
zpplot(zpthtemp)
```

```
% File: ...\Project\Appendices\Tools\System Identification
%          \Compensator.m
%
% This program is used for the RLOCUS design of the compensator in
% the z domain. This set of instructions were extracted
% from MATLAB demos (identification Toolkit).
%

Num=[  0    3.4621    7.1231   -5.6076];
Den=[1.0000   -1.7257    0.9669   -0.2175];

% System
HSys=tf(Num,Den,30);

% Integrator
CIntegral=zpk(0,1,1,10);

% Poles and Zeros first set
alfaNum1=-0.4;
betaNum1=-0.3;
NumC1=[1 2*alfaNum1 alfaNum1^2+betaNum1^2];
alfaDen1=.2;
betaDen1=0;
DenC1=[1 2*alfaDen1 alfaDen1^2+betaDen1^2];
Ctemp1=tf(NumC1, DenC1, 30);
rlocus(Ctemp1); zgrid, set(gca,'xlim',[-1.5 1.5],'ylim',[-1.5,1.5]);
pause;


% Poles and Zeros second set
alfaNum=-0.65;
betaNum=-0.2;
NumC=[1 2*alfaNum alfaNum^2+betaNum^2];
alfaDen=0;
betaDen=.2;
DenC=[1 2*alfaDen alfaDen^2+betaDen^2];
Ctemp=tf(NumC, DenC, 10);
rlocus(Ctemp); zgrid, set(gca,'xlim',[-1.5 1.5],'ylim',[-1.5,1.5]);
pause;


% Poles and Zeros third set
C1=zpk(.8,.1,1,10);
C2=zpk(-.8,-.2,1,10);

% Evaluation
HTot=HSys*CIntegral*C1*C1
rlocus(HTot); zgrid, set(gca,'xlim',[-1.25 1.25],'ylim',[-
1.25,1.25]);
[k,poles] = rlocfind(HTot)
pause
HClose = feedback(HTot,k);
step(HClose)
```

E.6. Visio

All the drawings presented below, are included in the VISIO stencil included in the attached CD EXCEL (`...\Project\Appendices\Tools\Visio\FerThesis.vss`).