Optimal Design & Operation of Biopharmaceutical Manufacturing Facilities Using a Rule Based Expert System Simulation Model

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Doctor of Philosophy

ASTON UNIVERSITY

January 1999

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SUMMARY

A simple analytical model of a fixed existing biopharmaceutical manufacturing facility is formulated based upon the assumption that balanced fermenter train operation yields optimal financial gain. Competing production schedules are evaluated using this model with the aid of two case studies; linear product and non-linear product accumulation. Commonplace heuristics promoting the maximisation of the production phase irrespective of fermenter train balancing are tested. A statistical study of the robustness of optimal schedules to batch failures is also presented.

An interactive graphical simulation model of the same manufacturing process is developed using an object-oriented design paradigm and rule based programming syntax. The general design of the simulation model is based upon differentiating unit operations into discrete processing activities. This simulation model is able to incorporate the finer operational details of the process, such as operator allocation, and to highlight process issues that are not easily accounted for by the analytical model.

A number of the possible operating schedules evaluated using the analytical model are re-evaluated using the simulation model. In the first instance, benchmark statistics, in the form of performance parameters, are determined for these schedules and process bottlenecks are determined. The flexibility of the simulation model allows a number of different process scenarios to be evaluated with regard to minimising the effects of bottlenecks. The robustness of the schedules to batch failures are also evaluated using the simulation model.

The results of the analytical and simulation model showed a high degree of agreement. The simulation results for the schedules studied show, generally, that those schedules with the balanced fermenter trains have the higher financial return.

Of the schedules studied using the simulation model the fed-batch schedule designated {1S2, 2P7} yields optimal operation. This also agreed with the analytical model results.

Keywords: Flowsheeting, Object-oriented, Rule based, Fed-batch cell culture, Batch failure analysis

ACKNOWLEDGEMENTS

I would like to acknowledge and thank all those who have helped me during the course of my studies at Aston:

In particular, my supervisor, Professor N.K. Slater for his enthusiastic support, guidance and availability during this time;

Dr. C. Marshall, co-supervisor at Glaxo-Wellcome, for her helpful advice and discussions;

The staff of the DCC facility at the Glaxo-Wellcome Beckenham site for their time and patience, particularly Scott Lorimar, Colin Cownley and Ian Duffield;

My family and friends for the support and encouragement they have given me;

and EPSRC (Total Technology Scheme) and Glaxo-Wellcome for sponsoring this work.

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Nomenclature

ACSL	Advanced Computer Simulation Language
AoN	Activity-on-Node
CAD	Computer Aided Design
CHO	Chinese Hamster Ovary
cGMP	current Good Manufacturing Practice
CPI	Chemical process Industries
DNA	Deoxyribonucleic Acid
EMEM	Eagle's Minimum Essential Medium
FCS	Foetal Calf Serum
Kb	Knowledge Base
KBES	Knowledge Based Expert System
MAb	Monoclonal Antibody
[MAb]	Monoclonal Antibody Concentration (MAb Units/l)
MINLP	Mixed Integer Linear Program
MIP	Mixed Integer Program
NSO	Proprietary Cell line Developed by CellTech UK
OO	Object Oriented
OOP	Object Oriented Programming
PAN	Process Activity Network
PFD	Process Flow Diagram
PGV	Principle Growth Vessel
QA	Quality Assurance
QC	Quality Control
USDoD	United States Department of defence

WHO World Health Organisation

d Number of Solera batch disposals to effluent during the repeat period, R (-).

d' =d/R ($days^{-1}$)

h Number of Production Vessel harvests during the repeat period, R (-).

P Duration of a Production Vessel cycle (days).

P' =P/R (-)

R Duration of the repeat period after which the pattern of Solera and Production Vessel operation is repeated (days).

S Duration of a Solera vessel cycle (days).

T Time required to turnround a Production vessel upon completion of a production batch (days).

Y The total financial gain which accrues from the manufacture of purified bulk product from harvests during the repeat period, R (£).

Y' The average daily financial gain which accrues from the manufacture of purified bulk product from harvests during the repeat period, R (£).

α The internal (company) value of purified bulk product which derives a single Production batch harvest (£ $batch^{-1}$).

α_0 Daily rate of accumulation of α for linear productivity of antibody (£ $batch^{-1}$ day^{-1}).

α_0 Specific value of antibody for non-linear productivity (equation 16), (£ M^3 g^{-1}).

β The variable cost of primary processing and purification for material from each Production batch harvest (£).

δ The variable cost of media and materials which is incurred by disposal of a Solera batch to the kill system (£)

ϕ The maximum duration of a Production batch (days).

κ Average number of Production batches up to, and including, the first vessel contamination (-).

τ The fixed cost per day of facility operation (£ day^{-1}).

μ Specific Growth Rate (hr^{-1})

K_s Monod Saturation Constant (gl^{-1})

K_la Volumetric Oxygen Transfer Coefficient

X_t Cell Density at time t (hrs)

X_0 Initial Cell Density (10^6 Cells/ml)

χ_{min} Minimum Seeding Density (10^6 Cells/ml)

T_{Cp} Total Cost of a Final Production Batch (£/unit of MAb)

V_{Cp} Total Variable Cost Incurred for a Final Product Batch (£)

F_{Cp} Total Fixed Cost incurred for a Final Product Batch (£)

M_c Cost of Preparing Nutrient Media (£/litre)

U_C Cost of Utilities Used (£/ unit of utility)

W_C Cost Incurred in Treating Process Waste (£/day)

Cl_C Cost of Materials used to Clean Fermenter Vessels (£/Vessel)

A_C Cost of in-process Testing (£/day)

L_C Cost of Process Operators for a Product Batch (£/day/Operator)

O_C Cost of Process Overheads (£/day)

Mt_C Cost of Equipment & General Maintenance (£/day)

FC_C Fixed Charges (£/day)

Eq_C Installed Equipment Capital cost (£/day)

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CHAPTER I

Introduction & Literature Review

1.0 Introduction

The concept and pursuit of optimisation forms the operating basis for every manufacturing, business and financial process, irrespective of the complexity of the process or the products involved. From the initial conception of a manufacturing or business process, questions as to how best to operate and manage that process are asked. There is a continual march towards doing things better than they were done before so becoming more efficient and cost effective.

Such a doctrine is particularly relevant to the biopharmaceutical industry, as it has begun to face new commercial pressures from the globalisation of world markets and economies. These pressures have lead to a greater emphasis on improving manufacturing facilities, better utilisation of existing capital assets, and delivering high value added products at costs acceptable to healthcare providers.

Two methods are evident in the optimisation of biopharmaceutical manufacturing processes. The first of these would require a series of real plant runs to evaluate the validity of possible operating strategies. Secondly, a virtual representation of the manufacturing environment could be developed using a suitable graphical modelling package. The latter option, not surprisingly, forms the basis of process optimisation studies in the biopharmaceutical industry.

Once suitable simulation models have been developed, existing processes can then be benchmarked in terms of appropriate process performance measures. Process bottlenecks can be highlighted and possible remedies evaluated rapidly without consuming real process resources. Assuming that

suitable graphical user interfaces are made available, then the simulation model can also be used as a safe training ground for operators. A fully defined and modelled process is easier to understand and talk about. Simulation can then help in communicating ideas across multi-disciplinary teams.

Software enabling the simulation of biopharmaceutical processes has only recently started to make an appearance on the market, while similar software for continuous processes, such as oil and gas processing, has been available for many years. A number of factors have hindered the development of bioprocess simulators. Such factors include the lack of physical and chemical property data for the vast range of biological products and the absence of appropriate unit operation models.

1.1 Original Research Objectives

The following presents an outline description of the specific research objectives of this work.

1.1.1 Objective 1

In the manufacture of biopharmaceuticals it is common to see process design heuristics that promote prolonged culture maintenance times. The reason behind this being that the longer the final culture is maintained the more active product that can be produced and final harvest titre is maximised. This process heuristic is generally proposed with no real consideration of the impact that fermentation vessel configurations and schedules may have upon the overall process economics.

It is shown that where production vessel schedules are chosen such that balanced or synchronised utilisation of fermentation vessels results, higher economic return is offered. Further, these balanced schedules do not in all cases correspond to longer production vessel duration and therefore

contradict the above stated process heuristic. This analysis is implemented through a simple analytical model assuming, in the first case, linear product accumulation and secondly, non-linear product accumulation.

1.1.2 Objective 2

The preferred method of investigating possible adaptations to existing processes with regard to process optimisation is to simulate these changes in a computer generated representation of the manufacturing environment.

It will be shown that a reliable manufacturing process simulation environment can be developed using an activity and process logic based approach to process simulation. This differs from the conventional approach to process simulation that employs complex unit operation and physiological process models. The advantage of an activity based approach is that simulation model development can be rapidly achieved with minimal programming knowledge. The direction of this work is to present a means of simulation design that is effective but also readily capable of compensating for gradual operational changes that take place over time in the real process.

The validity of the simulation model constructed is measured by carrying out process benchmarking studies using the simulation. The simulated benchmarks are then compared to the real process and to the results obtained from the analytical process model.

Additionally, the simulation will provide performance data on the utilisation of process resources. In this case the specific resource focused upon is that of process operators. Further, the facility exists to measure equipment and utility utilisation.

1.1.3 Objective 3

The results of the benchmarking studies reveal operational areas requiring optimisation. Routes to obtaining improved performance are examined using 'what if' scenarios. That is to say, specific alterations are made to the existing process, such as increasing the processing capacity of selected equipment items. The results of such changes are compared to the previous process benchmarks.

In this role the simulation model will provide a strategic analysis tool whereby different operating configurations, in the light of changing global market demands, my be evaluated without risking real process resources and time.

1.2 Thesis Outline

Chapter II describes a simple analytical model of an existing monoclonal antibody manufacturing facility owned and operated by the Glaxo-Wellcome group. A techno-economic model is developed and used to determine the performance of competing process operating strategies and vessel operation schedules. An analytical model of process tolerance to batch failures is also introduced.

Chapter III gives a brief introduction to the primary research tool used, i.e. the software system used to extend the analytical model of Chapter II to the development of an interactive dynamic simulation model. This chapter also embarks upon an explanation of the basic concepts underlying the final version of the simulation model.

Chapter IV presents simulation data on a range of benchmark process statistics for two competing process strategies and vessel operation schedules there in. The results from the simulation runs are compared against the predictions from the analytical model of Chapter II and also used to elucidate operational areas requiring further analysis such as debottlenecking.

Chapter V presents the results from a number of *'what if'* simulations carried out to evaluate optimal operational conditions for those areas identified in Chapter IV .

Chapter VI contains a final discussion, conclusions and recommendations for further work.

1.3 An Introduction to Biopharmaceutical Manufacturing Processes

The biopharmaceutical industry may be seen as one of the many facets of the biotechnology industry in general. Although it is often thought of as new technology, biotechnology is one of the oldest industrial technologies with a recorded history dating back to biblical times[1]. To the biochemical engineer, the intentional use of micro-organisms to produce beer, wine and cheese is biotechnology. To this list the biological treatment of sewage and waste wasters has also been added. The term biotechnology was first coined by Ereky, in a book published in 1919, to describe all lines of work by which products are produced with the aid of living organisms[2] .

At about the same time an industrial process for the mass production of acetone by fermentation had been developed. This process very much fitted into the definition provided by Ereky. From about this point in time, biotechnology became synonymous with industrial fermentation technology and as such was defined as so in the Journal of Biotechnology and Bioengineering in 1962.

The basic definition from Ereky remained unchanged until in 1979 when the word was redefined for a genetic engineering journal to describe scientific and financial developments in the field of genetics[3] .

The key feature of industrial biotechological processes is that they exploit living organisms to produce commercially viable products, regardless of whether those organisms are genetically manipulated or naturally occurring.

Although a general and accepted definition of biotechnology exists, there is however a significant distinction between the products of traditional fermentation processes and those of processes employing genetically manipulated organisms. Webb and Atkinson[2] refer to these processes as 'traditional biotechnology' and 'new biotechnology' respectively. In the case of the former, the products are generally of low to medium value and are produced in quantities requiring large scale processing equipment. The products of the latter are often of extremely high value and produced in minute quantities. Examples of these products are shown below in Table 1.1 .

CATEGORY	ACTIVITIES
High volume, low value	Methane, ethanol, biomass, animal feed, water purification, effluent and waste treatment
High volume, intermediate value	Amino and organic acids, food products, baker's yeast, polymers
Low volume, High value	Antibiotics and other health care products, enzymes, vitamins, monoclonal antibodies

Table 1.1 Classification of some of the products of the biotechnology industry.

The low volume, high value end of the product spectrum has, since the mid 1970s, been increasingly dominated by the production of monoclonal antibodies via animal cell culture systems. Early interest in these products envisaged their production via bacterial and yeast systems to take advantage of the commercial experience already gained with these systems. However, a number of difficulties have restricted the use of these cells as host[4]; these include:

1. many of the protein products of interest are glycosylated. This complex step is not easily achieved with bacterial cell lines.
2. post translational modifications confer the crucial conformation and the desired biological activity on these proteins. This is another step not easily achieved by bacterial or yeast host cells.
3. the desired protein is not usually secreted in these systems and therefore can entail complicated recovery practices.

The history of animal cell culture systems as applied to manufacture of products of therapeutic value can in some ways be traced back to 1798, with the pioneering work of Sir Edward Jenner. Jenner was able to show the protective efficacy of the material emanating from the cow pox pustule in protecting humans against the disease of smallpox. This work laid the foundations for the eventual global eradication of the disease of smallpox; achieved according to the declaration issued by WHO (World Health Organisation) on 9th December 1979[5].

In Jenner's case, the animal cell systems that were used to provide the therapeutic agent were suitably infected farm yard cattle. Gradual advances over time in the understanding of the cellular basis to living organisms led eventually, in the early 1900's, to the first *in vitro* maintenance of animal cells by Ross Harrison[6]. This initial work became known as the 'Hanging Drop' technique and is illustrated in Figure 1.1. Harrison suspended dissected nerve tissue from frog embryos in lymph fluid which was allowed to clot as a droplet on the underside of a microscope cover slip. Figure 1.1 shows how this was mounted on a hollow microscope slide and sealed with wax. This method allowed Harrison to observe the growth of the embryonic nerve cells for several weeks.

Further advances upon the technique and materials used prolonged the life span of the animal cells used and enabled the use of a wider range of mammalian cells. Particularly, advances in aseptic handling techniques contributed to the extended cell life spans that were being witnessed. Much of

the research effort at this time was focused upon maintaining the viability of animal cells in artificial environments.

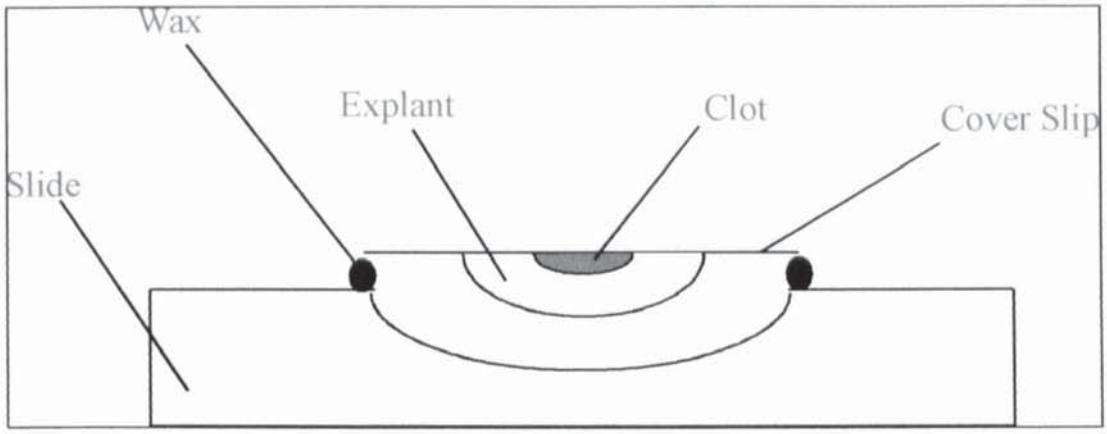

Figure 1.1 Harrison's hanging drop technique (1907).

Flemings original discovery of penicillin in 1929[7] and other subsequent discoveries of anti-infective agents led ultimately to a major breakthrough in animal cell culture technology during the late 1940's, when antibiotics (penicillin and streptomycin) became widely available. The addition of antibiotics to cell culture medium made the handling of such cultures less prone to infection and contamination.

The 1950's witnessed major developments in the analysis and interpretation of the nutritional requirements of cultured cells. This work was predominantly attributed to Earle and Eagle[6]. They defined a formulation of chemical compounds required to replace the growth promoting biological fluids previously used for cell growth. The advantages of this formulation, known as Eagle's minimum essential medium (EMEM), was that it was well defined and therefore provided for greater consistency between batches, more easily sterilised and thus lessened the chance of contamination. A breakdown of EMEM is provided by Lambert and Birch[8]. However, this formulation and its derivatives required an additional supplement of chemically undefined blood serum to provide unidentified growth factors and hormones.

Manufacturing processes employing animal cells have always been at a cost disadvantage in comparison to processes based upon microbes[9]. Among the reasons for this is that animal cells require complex and expensive media. The basic cell culture medium consists of 30 to 50 ingredients. This ingredients list is supplemented by the addition of serum and antibiotics. The cost differential between animal cell and bacterial processes is illustrated in Figure 1.2 below. From this it is seen that the medium for animal cell processes can be as much as 15 times more costly than for bacterial processes.

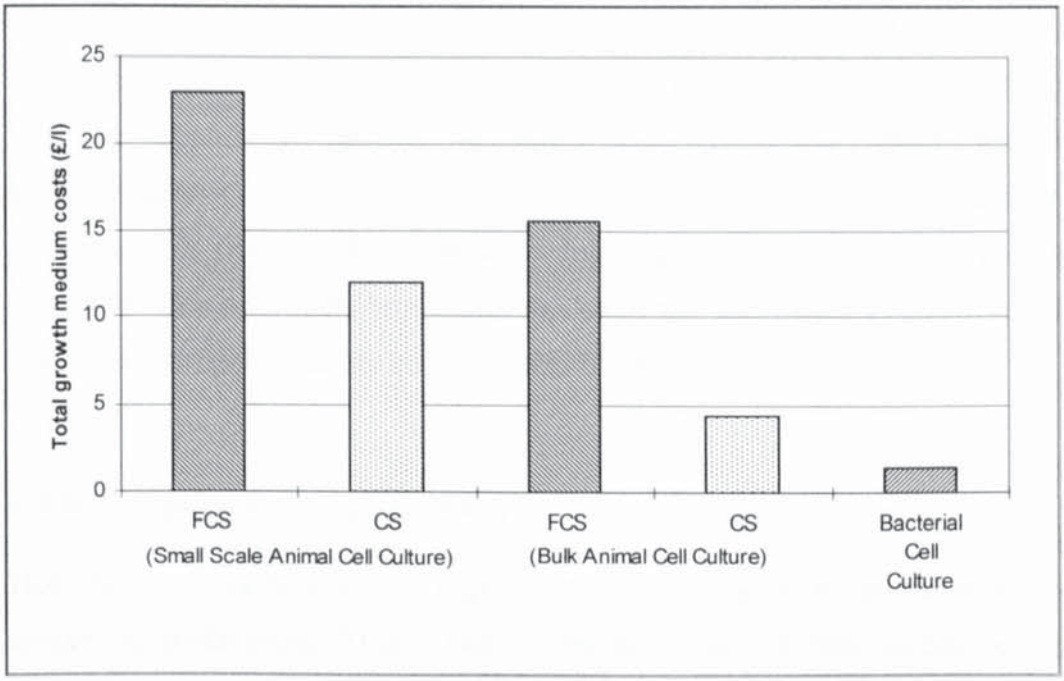

Figure 1.2 The total cost of growth medium for a small scale animal cell culture and bulk animal cell culture process using two serum types (FCS = Foetal Calf Serum, CS = Calf Serum). Both animal cell processes are compared with the typical total culture medium cost for a bacterial cell culture process.

The bulk of the observed difference can be attributed to the cost of the serum and antibiotics required. Significant cost reductions may be achieved by the omission of antibiotics as long as there exists enough confidence in the aseptic techniques of laboratory staff. Further importance is placed upon staff training since many mycoplasmal and viral contaminants cannot be readily countered using antibiotics. Human expertise is an essential part of any cell culture operation. However, people are walking animal cell cultures; they

provide a good substrate for micro-organisms and are hence vectors of contamination. There is therefore a great deal of responsibility upon staff to maintain the right attitude to such work in order to assure success[10].

Serum has been intensively studied in an effort to dispense with it in the view of its high cost and the fact that it is undefined, of variable quality, and often the source of microbial contamination. Modern day concerns regarding the use of serum supplements have been highlighted by the outbreak of bovine spongiform encepthalopathy (bse) in the United Kingdom. The infectious agent in this case is stable and occurs in the nervous tissue of bovines for the most part although this does not exclude its occurrence from other tissues. Thus it is held unlikely that serum is effectively contaminated with this agent but the possibility cannot be ruled out. Concerns about this and contamination of serum with other infectious agents have lead to extensive developments of cell culture media systems that do not use serum. The draw back with this approach has generally been the high cost of such ventures and also the added quality control costs to any cell product.

1.4 Monoclonal Antibodies (MAb)

The immune systems of all higher animals are able to produce proteins known as antibodies. These play a dynamic role in the recognition and immobilisation of foreign compounds (known as antigens) to bring about an immune response and prevent infection within the living system.

The usefulness of antibodies outside living systems relates to their highly specific binding ability to particular compounds. Prior to 1975, attempts to isolate antibodies from an animal system resulted in a heterogeneous mixture which depended upon the previous antigenic exposure of the animal. Such mixtures are referred to as being polyclonal. In 1975 Kohler and Milstein developed a technique to produce cells capable of continuous secretion of a single type of antibody (monoclonal) to a predefined antigen[6,11,12,13] . These cells are referred to as hybridoma cells. This technical milestone laid the

ground work for a technology capable of producing kilogram quantities of antibody to an antigen of choice. This has lead to an increasing number of applications in diagnostics and therapeutic drug areas. Spier[14] quotes a survey between 1983 and 1986 where some 40 different products of animal cells are identified. Table 1.2 lists just some of the therapeutic products derived from animal cell culture systems.

Animal Cell Derived Products
Growth factors: Nerve growth factor, Epidermal growth factor
Blood factors
Monoclonal antibodies
Interferons
Vaccines: Foot and Mouth Disease Vaccine (FMDV)
Proteases: Urokinase
Hormones: Human growth hormone, Insulin, Calcitonin, Parathyroid hormone

Table 1.2 List of products derived from animal cell culture systems[15].

Product application has a great baring on the choice of manufacturing process both with respect to scale and quality requirements. While diagnostic antibodies may be required in tens or hundreds of grams per year, some therapeutic antibodies may be required in tens or hundreds of kilograms per year. Additionally, the purity requirements for therapeutic antibodies are far more exacting than for an *in vitro* diagnostic antibody. This is exemplified by Cole[16], who describes the pharmaceutical industry as being unique in the procedures and methods of manufacture that it uses to ensure the integrity of the products it produces. This is effectively achieved by three main functions: current Good Manufacturing Practice (cGMP) regulations[17], Quality Assurance (QA) regulations and Quality Control (QC) regulations.

The quality standards as laid out in cGMP require that the process be contained, that cross-contamination is avoided, that pure water is used in the manufacturing process and that air is filtered and conditioned. cGMP also requires that staff be carefully selected and trained. The design of the actual

processing equipment used is also a key factor in the success of any manufacturing facility. Particular attention is paid to the ability to clean and sterilise fermenter vessels and purification equipment. Where facilities are to be used for making different products, special attention is paid to the operating procedures, segregation of operations, such as cleaning, and the avoidance of cross-contamination.

In comparison to chemical pharmaceuticals, biopharmaceuticals are complex molecules, often showing unavoidable microhetrogeneity[11, 18]. As a result there is a greater emphasis upon controlling the process rather than relying upon an analysis of the final product[19]. During the development phase of such processes, process validation in terms of identification of impurities and contaminants is addressed. This leads to the initiation of clearance studies. Process validation is used to stress the process and demonstrate its robustness and reproducibility.

1.4.1 Animal Cell Culture Systems for Monoclonal Antibody (MAb) Production

Many MAbs are produced by growing cells as tumours in mice or rats. Typically the MAb concentrations in such cases reach about 10 g/l. However, for therapeutic MAbs, manufacturers have adopted *in vitro* cell culture techniques. This approach reduces the possibility of introducing adventitious agents and irrelevant antibodies from the host animal. There has also been some ethical reasoning behind this choice, as it reduces the unnecessary use of animals in research and development.

1.4.2 Animal Cell Culture System Models

Understanding the roles of physiological and environmental factors on the growth and metabolism of animal cells is a prerequisite for the development of rational scale-up, control and optimisation procedures. Models of animal and microbiological systems are generally classified as unstructured or structured[20]. The current bias has been towards the use of unstructured

models because the numerical solution techniques and computational power required for solving structured models is somewhat prohibitive.

An unstructured model assumes that only a single variable such as cell number or dry weight is sufficient to describe the biosphere. A structured model allows the individual nature of the entities which make up the biophase or to any variations in the quality of the biophase to be accounted for. Structured models can be further divided into those that are chemically structured and those that are nn-chemically structured. Chemically structured models consists of real and measurable components such as DNA, RNA and proteins. A non-chemically structured model recognises that in a pure culture the biosphere consists of cells of different cell sizes and ages and the biosynthetic capabilities of the a cell depend upon age or size.

A further taxonomy arises from the recognition that cell cultures consist of distinct cells or entities. A segregated model explicitly recognises that a population consists of individuals each of whom may have distinct properties.

The general trend, as already stated above, is that when it comes to cell growth and product formation models unstructured non-segregated formalisations are preferred. The models proposed by Miller and Blanch[21] and Bree *et al* [22] for the determination of the specific growth rate of cultured hybridomas provide an example of two examples of the conventional unstructured formulations. In either case a modified version of the Monod equation is used.

The Monod equation is one of the simplest types of unstructured and non-segregated model and defines the relationship between specific cell growth rate and an essential compound's concentration. The general form of the Monod equation is given below.

$$\mu = \frac{\mu_{max} S}{K_s + S} \qquad (1.1)$$

where μ is the specific growth rate (hr^{-1}), μ_{max} is the maximum specific growth rate (hr^{-1}), K_s is the Monod saturation constant (g^{-1}) and S is the concentration of an essential compound (g^{-1}).

The equation used by Miller *et al* has the form:

$$\mu = \mu_{max}\left[\frac{C_C}{C_C + K_C}\right]\left[\frac{C_N}{C_N + K_N}\right]\left[\frac{K_A}{C_A + K_A}\right]\left[\frac{K_L}{C_L + K_L}\right] \qquad (1.2)$$

where C_C, C_N, C_A, C_L are the extracellular concentrations of glucose, glutamine, ammonia and lactate, respectively. Monod saturation constants for glucose and glutamine are expressed as K_C and K_N, respectively, and the inhibition constants for ammonia and lactate as K_A and K_L, respectively. However, Miller *et al* make no effort to use this formulation to simulate dynamic changes in growth rate over the course of a batch and continuous culture. The formulation used by Bree *et al* mirrors that of Miller *et al*; however, there is no dependence upon glucose.

Bree *et al* use their simulation model to predict hybridoma growth in a batch culture using a set of differential equations to describe the dynamics of both viable and non-viable cell concentrations and antibody concentration during the process. The simulation results and pilot plant data in this correlated well, except for the prediction of lactate concentrations. This discrepancy was accounted for by an over-simplification in the assumed role of lactate in the model system.

Batt *et al*[23] present a structured model based upon hybridoma metabolism. The known details of the metabolic processes in the hybridoma cell line used are incorporated by dividing the cell mass into four intracellular pools. Each pool is defined by differential equations that are identical for both batch and continuous hybridoma cultures. The four pools defined in this case are amino acids, nucleotides, proteins and lipids. Product secretion is also defined by

dynamic equations for antibody, lactate and ammonia. The simulation model output is compared against the experimental results presented by Miller *et al.* The results in this case correlated well and add greater validity to the use of structured models.

Further, more recent applications of structured segregated models by Faraday *et al*[24] have shown the use biological age distribution within a cell cycle framework as a predictive tool for viable hybridoma cell concentrations.

Product formation kinetics is generally defined as being either growth associated or non-growth associated[25]. For monoclonal antibody production a combined growth and non-growth associated model attributed to Leudeking and Piret[26] is often used. The results of Miller *et al* show that for batch culture of hybridomas antibody production is partially growth associated, while for continuous operation the reverse was found to be true. Bree *et al*, however, assumed a non-growth association for their work, while Batt *et al* also assumed a non-growth association for antibody production. The assumptions of the latter two seem to be have been justified by the work of Renard[27] and co-workers who showed that antibody production is non-growth associated or is due to a constant secretion rate from viable cells.

One explanation offered for the observation of Miller *et al* for a batch culture was that as culture conditions in batch systems are constantly changing, the values obtained cannot be totally reliable.

1.5 In vitro Production Systems

Over the last decade or so a number of design concepts have been proposed and developed with varying degrees of success. These approaches have ranged from suspension cell culture - with and without an airlift provision to improve oxygen transfer - to the attachment of the cells to a variety of matrices. However, only a very few of these designs have the real potential for an economical large-scale operation.

Merton[28] presents a general classification of the available bioreactor configurations along with some relevant performance data for the configurations presented. While more specific reviews based upon the modes of operation for reactor vessels are provided by Reuveny and Lazar[29] for batch wise stirred tank operation, Shevitz *et al*[30] for perfused stirred tank operation, Birch *et al*[31] for fed-batch airlift operation, Applegate and Stepanopoulos[32] for perfused ceramic matrix operation and Altshuler *et al*[33] for perfused hollow-fiber operation.

1.5.1 Mode of Operation: Batch, Fed-batch & Perfusion

The large scale cultivation of animal cell systems is generally performed via one of three operating modes. These are batch, fed-batch or perfusion operating modes[4, 15, 34].

A batch culture is a closed system in which cells are inoculated into a quantity of nutrient medium contained in a suitable vessel. The culture is incubated at the required temperature and events are then allowed to run their course. Therefore, in such a system, no additional nutrients are replenished except oxygen and the cells in a batch culture are subject to a constantly changing environment in which nutrients are depleting and waste product concentrations are increasing. This situation leads to the inhibition of cell growth and/or product formation due to nutrient limitation or to the toxic accumulation of waste products[15, 34, 35].

A fed-batch culture, on the other hand, has regular additions of nutrient media. Such bolus additions of nutrients has the effect of maintaining a relatively constant nutrient concentration and limiting the concentration of waste products. The resultant effect is that higher final viable cell densities and MAb concentrations are achieved in comparison to conventional batch processes, as is exemplified by the following. Viable cell and MAb concentrations of approximately $1x10^6$ cells/ml and 200 μg/ml respectively for

fed-batch operation have been reported in comparison to $0.2x10^6$ cells/ml and 100 μg/ml for the same cell line employing batch operation[25, 36]. In this case there is a five fold increase in the viable cell density over the batch culture and a doubling in the MAb concentration for the fed-batch culture over the batch culture.

In perfusion cultures, cells are retained by some mechanical means, such as a dialysis membrane or filter, which permits the exchange of spent medium with fresh medium outside the culture. Both the nutrient and waste product concentrations can be controlled by varying dilution rate of the system. However, because of the intense use of nutrient medium, perfusion systems can be less cost effective, particularly if the medium used consists of serum[15, 35].

1.6 Optimisation of Biopharmaceutical Manufacturing Processes

Simply put, optimisation is about manipulating the characteristics of a given system to obtain the best possible performance from that system. The performance of the system is measured as either the minimisation or maximisation of the characteristic of interest. As to whether the objective is to minimise or maximise is determined by the nature of the characteristic itself. That is, if the characteristic of interest is the total production cost, then the objective would be to minimise this, while for productivity the objective would be to maximise it. Generally speaking the characteristics that are to be minimised or maximised are usually dependant upon other characteristics of the system. Hence, it is these secondary characteristics that must be manipulated to achieve the desired result[37, 38].

For example, determination of the optimal nutrient recipes such that the final product or biomass yield is maximised fits into the above secondary characteristic definition. That is, increasing the yield of product implies a greater productivity and a greater productivity leads to a lower total cost of production. Similarly, studies into optimal designs for reactor/fermentation

vessels have concentrated upon optimising the environmental conditions. Lavery and Nienow[39] studied the effects of basal and growth medium on the volumetric oxygen transfer coefficient (K_la) and also the effect of antifoam. The presence of antifoam was shown to reduce (K_la) by as much as 50%. The relevance here is that oxygen concentration has critical effect upon cellular metabolism which not only affects growth but also the products that can be expressed by the cell[23, 40]. However, the removal of antifoam additives may result in entrapment of otherwise viable cells within foam layers and cell damage due to shearing effects within collapsing foam structures[41]. Even the cell lines themselves are optimised via genetic manipulation to maximise expression of the desired final product. All of these secondary characteristics have a significant impact upon the final productivity of a process.

The examples given thus far represent optimisation at one level only; predominantly physiological in nature. Another, more global approach is to consider the physical manufacturing process and specifically the relationship between equipment items in terms of scheduling material and resource flows between them. Optimisation in this case relates to minimisation of equipment idle times, minimising transfer delays and generally debottling and increasing the operability of the process.

Literature in the field of design of entire biochemical processes is fairly scarce, particularly where optimal design and operation are concerned. The majority of the work published in the area of biotechnology and biochemical engineering centres on the optimisation of individual unit operations through the development of better mathematical models[42].

Because of the high value of biopharmaceuticals, like monoclonal antibodies, concerns about optimal process operation have generally tended to be side-lined, while speed to market with such products has had greater emphasis put upon it[43]. Increased global competition, greater regulatory pressures and increased research and development time and costs have prompted the biopharmaceuticals industry to explore means of better capital utilisation[44, 45].

Only those biopharmaceutical manufacturers that are best able to improve operations of their existing facilities will gain a significant competitive advantage[46, 47].

Although the literature dealing specifically with total process optimisation for biopharmaceutical facilities is very limited a considerable degree of interest has arisen in the optimal operation of batch processes in general[48, 49, 50, 51, 52]. The particular relevance in this case to biopharmaceuticals manufacture comes from the fact that predominantly all high value biopharmaceuticals are produced via batch or fed-batch (semi-continuous) processing[42, 53].

Batch processing has received particular interest because, for low volume high value products, it offers an efficient and flexible manufacturing strategy[48-52, 54]. Further, batch processes in general have an advantage over continuous processes in that they are better suited to cope with variability in raw material supply and in product demand[55]. Parakrama[56], in a survey of 99 batch processes operated by 74 UK manufacturing concerns, cited overcapacity through the inability of high volume continuous processes to cope adequately with variable product demand as a possible reason for the resurgence of interest in batch processes. This survey showed that 80% of the plants are producing chemicals in steady or growing markets, and that only 6% of the processes were likely to be converted to continuous operation, despite the fact that 95% of the processes were capable of such a change. Although the speciality chemicals sector was taken as the basis for this survey, the general observations are still applicable to the biopharmaceuticals manufacturing sector in highlighting the strengths of the batch processing paradigm.

The primary criticism of batch processes has been their lack of reproducibility and resultant fluctuations in product quality. This problem has been addressed to some degree by the implementation of automated control systems[57]. For biopharmaceuticals manufacture, final product quality is an extremely important issue with regards to obtaining and maintaining regulatory approval and licensing. To aid in minimising product quality

variation rigorous operating procedure protocols are established and documented in accordance with cGMP, QC and QA.

Batch processes have traditionally been linked with multi-product or multi-purpose capability due partly to the ease of reconfiguration of the standard equipment types used[58, 59]. In a multi-product facility all products follow the same sequence of processing steps. In a multi-purpose facility, products may follow different paths through the facility dictated by the particular processing needs of the product[59]. To use the current scheduling terminology, a multi-product facility is known as a flowshop and multi-purpose facilities as a jobshop[60]. Operating flowshop or jobshop facilities places heavy demands upon the available resources and therefore requires competent scheduling to ensure that unnecessary delays are prevented and product delivery deadlines are met[61, 62, 63].

The particular attraction of either of these two facility configurations for biopharmaceuticals manufacture arises from the possibility of reducing new facility development time and costs and thereby facilitate a rapid market entry for new products. If an exiting facility could be used for the manufacture of a new product or products, then the need to construct new purpose built plant is removed. This has its obvious capital savings, but also having existing plant capacity available before phase III trials are completed can save at least four years on product development time[64].

A major regulatory concern in such cases would be to prevent cross-contamination between product lines[65]. With a flowshop configuration temporal separation between product lines would have be maintained, while for a jobshop configuration with simultaneous product processing spatial separation is required.

Samsatli *et al* [42, 66] present a mathematical programming based optimisation of process design and scheduling for a simplified biochemical process consisting of a sequence of unit operations. The general approach used in

this case was to break the overall problem down into sub-problems consisting of mixed integer non-linear programming (MINLP) problems. Optimisation problems involving both continuous and integer variables are known as mixed integer problems (MIPs), where the integer variable may take only binary values (0 or 1). These are used to represent different process alternatives, such as a holding tank is available or it is not available[67].

For already existing biopharmaceutical manufacturing processes, successful total process optimisation is complicated more often by operational specifics rather than the number of products. Large scale animal cell based processes employ a production configuration which involves a Principal Growth Vessel (PGV) feeding a defined fraction of its contents to one of a number of production vessels. Both the PGV and the production vessel are charged with sterile nutrient media, so that the PGV can begin another growth cycle and the production vessel to begin producing the final crude product. However, the duration of the PGV and the production vessel are, in most cases, not identical. The PGV duration is dictated by the requirement to reach a maximum cell density suitable to inoculate the production vessel, while the production vessel duration is dependant upon reaching an adequate harvest titre. As a result of the different requirements a temporal imbalance exists between the PGV and the production vessel.

Such an operational phenomena has a number of consequences for the processes as whole that have to be reconciled with process performance objectives in mind. For example, out of phase operation can result in a number of PGV batches being disposed to drain because no production vessel is available to accept it. The opposite of this situation may occur where the production vessel is ready and available to accept a batch of inoculum from the PGV, but, the PGV is not yet ready to initiate a transfer. This results in an idle or non-productive time for the production vessel concerned.

Further, such an asynchronous characteristic can produce significant variations in the utilisation of other equipment items and processing stages. As an example, nutrient media preparation is considered. When both the PGV and the production vessel are in phase, a high demand for nutrient media is placed upon the media preparation stage of the process for both vessels. The design capacity of the media preparation stage will be such that it is never exceeded (although this may not be true for new products introduced onto an existing manufacturing facility). However, once the PGV and the production vessels are out of phase, the media demand profile changes and the overall media preparation stage utilisation profile will then oscillate between a low utilisation and near maximum utilisation.

Attempting a total process optimisation for such cases clearly encompasses a number of issues of operational importance. Optimisation of a process with regards to a number of performance parameters becomes difficult. The mathematical analysis becomes involved and often intractable, leading at best to sub-optimal solutions. As a result, it is usual to select a single process performance parameter for optimisation, usually an economic performance indicator such as the total product cost of the process.

The preceding sections show that undertaking total process optimisation studies requires a great deal of information about how the process of interest works. This information is used to construct mathematical unit operation models. These combined models effectively form the basis of the total process model. Scheduling considerations in such cases can be accounted for by defining relative equipment priorities and cycle times. Using computer software, such process models can be used to drive real and faster than real time[68] graphical interactive simulations of the process. By making the process simulation interactive the possibility of introducing 'what if' scenario analysis exists[68, 69]. Further, an interactive simulation model provides a safe process operator training ground.

1.7 Introduction to Process Simulation

The word simulation means to replicate the particular conditions or behaviour of a situation or system by means of a single model or a number of interacting models. These models are often mathematical in nature and the solutions can either be analytical for the simple ones or as is becoming increasingly common, numerically based.

The reasons for developing and applying simulation models can be varied. However, generally simulating the behaviour of a system of interest is cheaper than studying the real thing. Often simulation is the only way of studying the system. Also depending upon the system of interest, simulation can be a safer option, providing a risk free environment in which to test out hypotheses. For example, during World War II simulation studies of bombing strategies were carried out to investigate their effectiveness[70]. The level of the technology available to simulate such systems is demonstrated by the fact that humans played the roles of aircraft in these simulation studies.

The post war years saw a boom in the computational hardware and software available to develop simulations. The software, which at the time meant programming languages like Fortran and Advanced Computer Simulation Language (ACSL) made the development of complex simulation models possible. However, the procedural nature of these languages made the modelling of real systems with parallel operations difficult. This led to the United States Department of Defence (USDoD) funding the development of new programming languages. The very involvement of USDoD showed the level of importance to which simulation studies had reached.

The last two decades have seen a major leap in the availability and sophistication of both computer hardware and software. It was during this period that the personal computer (PC) was born. The PC represented low cost and high power computing for the masses. The PC facilitated the transfer of powerful simulation packages from mainframe computers on to small desktop machines.

Although computers and process simulation had been used in the chemical process industries from as early as the 1950's[71] the same is not true for the biochemical process industries. This is particularly surprising considering that the biotechnology industries has a recorded history pre-dating that of the chemical process industry. It would therefore be assumed that a greater body of knowledge in the form of mathematical formalisations would have evolved to define the behaviour of such processes and make them amenable to process simulation. However, custom built simulations for biochemical processes have only recently become available[69]. Early generations of simulation software for biochemical processes were basically just standard chemical process flowsheeting packages.

The question that arises is why has the development of simulation software specifically for biochemical processes lagged so far behind that of the chemical processes. The general consensus points to a considerable gap in the understanding of chemical and physical characteristics of the materials used in biochemical processes and also in the lack of suitable mathematical models to predict process phenomena such as mass and heat transfer and fluid mixing for biological systems. Even when suitable models and characteristic chemical and physical data do exist they are often process specific. This makes the development of a useful generic bioprocess simulator a difficult proposition.

The development of a general purpose biochemical process simulator is hindered by the current reliance upon a model based approach to simulation. In an effort to overcome some of these problems, knowledge based expert systems (KBES) have been applied to the development of simulators. KBES use the concept of captured process knowledge stored as a series of general rules to provide reasoning ability about the process of interest.

1.7.1 Benefits of Simulation

Being able to simulate complex real systems has many benefits. Some of these benefits are echoed in the following quote.

"*Make sure that you take the Star Tours ride when you visit Disneyland. It's as good as the real thing, and a lot cheaper!*"[71]

Simulating a particular system has cost advantages. Clearly, simulating a journey through the Solar System would be cheaper than building and operating a 'Stella Cruise Shuttle'. Similarly, applying this principle to process design and operation allows hypothetical processes and operating practices to be investigated without real resources being consumed.

Simulation also has safety implications. The provision of an environment, through simulation, that accurately mimics the behaviour of a real world system means that the risks or dangers of that system may be experienced. This is particularly important for process operator training purposes.

Consider, for example, operators within a nuclear power plant. It is essential that each operator be able to cope with emergency situations. The majority of such power plants are designed with multiple automatic fail safe and backup systems. This takes a great deal of pressure away from operators. Using simulations of such processes it is possible to induce multiple safety system failures. The operators are then able to familiarise themselves with emergency protocols, without any real risk being encountered.

1.7.2 General Concepts of Process Simulation

To simulate a given process, a basic requirement is that of a mathematical model and a means of solving that model. A simulation of a particular process environment may consist of a single model or a number of models.

A model, for process engineering purposes, may be defined as a mathematical representation of the behaviour of a real process or various elements of that real process.

For example, a simulation of a chemicals manufacturing process will consist of models defining specific unit operations. Typically, such a process will be made up of several different unit operations. Therefore, any simulation will consist of a similar number of unit operation models.

Once models have been developed and validated they are generally used in two ways. These are the design solution and the performance solution[71, 72]. In the design solution, the input and output values are known. A solution to this model results in the values of the design parameters. The performance solution determines the outcome from a given set of operating conditions and initial inputs. Figure 1.3 and 1.4 illustrate the concept of the design and performance solution respectively.

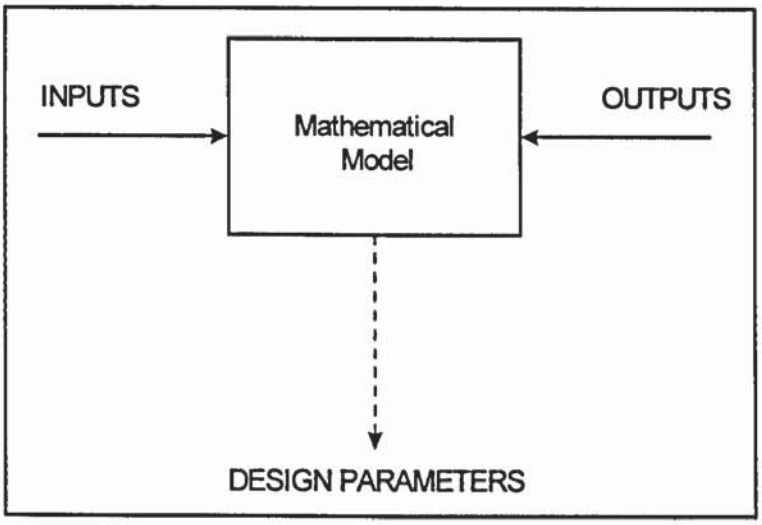

Figure 1.3 Mathematical model used to obtain design parameters (adapted from [71]).

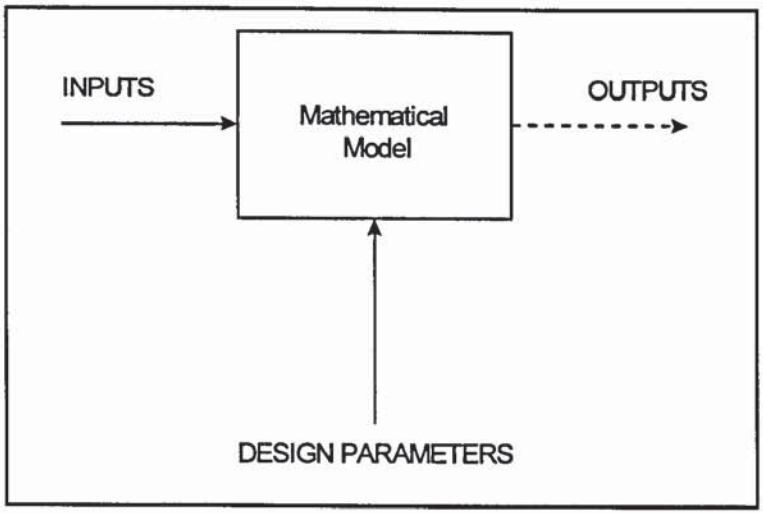

Figure 1.4 Mathematical model used to determine performance (adapted from [71]).

The two approaches to the application of process models that are currently used for simulators are those of the sequential modular and the equation-oriented approaches.

The sequential modular approach relies upon a step by step advancement of the simulation. For this reason it is particularly suited to processes with a number of unit operation connected in series. Applying a model for one unit

operation produces the outputs values that are used as inputs for the next unit operation model.

The equation-oriented approach relies upon gathering all the relevant equations into a global system model and solving them simultaneously. This approach will generally be more demanding in terms of the numerical techniques required to reach solutions. However, equation-oriented methods are highly flexible in terms of the type of system to which they can be applied.

The present market for steady-state process simulators consists mainly of those simulators based upon the sequential method. Examples of commercially available computer-aided design (CAD) programs include PROCESS, ASPEN, HYSIS, HYSIM, ChemCAD and SPEEDUP[73]. Most of these now have added 'intelligence' aimed at minimising the need for iteration and speeding up convergence to a solution[71].

1.8 Simulation in the Process Manufacturing Industry

Within the process manufacturing industry the use of process simulators has become more prevalent. Computers have, however, seen use as early as the 1950s within the Chemical Process Industries (CPI) to solve mathematical models[74, 75]. This rise in the use of simulators is attributable to a number of factors. Chief among these reasons would be the increased power and reduced cost of the hardware and the increasing sophistication of the software[76].

Further, process operators and managers are continually under pressure to increase process efficiencies through process modifications, conserve energy and material resources, size equipment and study the effect of variation in the design and operating parameters on the performance of processes. The only feasible method available to address these 'what if' type questions accurately is that of process simulation[74].

Simulation software has traditionally required that the user have expert knowledge and experience of the both the real process and the mathematical representation of the process. However, the power, flexibility and usability of modern simulation software means that even relatively inexperienced users can develop solutions rapidly[77].

The type of simulators used by the process manufacturing industry can be divided into two types. These are continuous and discrete event simulations. Continuous process simulation is generally applied to continuous chemical manufacturing processes. While discrete event simulation is applied to batch manufacturing processes.

A continuous process simulation model will be concerned with the evolution of a system over some chosen quanta of time (Δt). In effect, the state variables of such a system may change continuously. Mathematical representation of continuous systems is generally achieved via differential and algebraic equations.

Discrete event simulation relies upon being able to describe a given process as a number of activities with a discrete set of possible states and defined start and end times for those activities. Transitions in the state of that activity are observed only at discrete points in time.

For example, consider the filling of a vessel. The possible states of that vessel being empty, full or overflowing. The transition from any one state to another is only observed at discrete points in time. These state transitions are associated with allowed events[78] and hence define a discrete event system. The event in this case may be regarded as a driver of change from one state value to another. Therefore, an event is identified with a specific action taken or the occurrence of several conditions which are all suddenly met to result in a given state[79].

1.8.1 Process Simulation in the Bioprocess Industry

Only recently has the software necessary to simulate biopharmaceutical processes and bioprocesses generally become available[69]. The interest in bioprocess simulators has been sparked by many of the recent advances in biotechnology that have great commercial potential[80]. Realisation of the commercial potential of these processes and products is extremely dependant upon process development studies. The only feasible means available to explore process development issues is through process simulation. A reliable simulation model of a biopharmaceutical manufacturing process can be helpful in persuading regulators who license a product according to the process used to make it[69].

The techniques used for the simulation of traditional chemical processes can, in theory, be applied to biochemical processes. Since both process types will consist of a number of unit operations, mathematical models are definable for each unit operation. The unit operation models will themselves be constructed from a number of mathematical models predicting process phenomena, such as mass and heat transfer and fluid mixing characteristics. For traditional chemicals manufacturing facilities these models are generally well defined and understood and so form the basis of chemical engineering teaching.

However, simulating bioprocesses is plagued by a number of significant problems. These include:

1. the large diversity of biological products, widely differing in chemical and physical properties;
2. most biological products, especially the high value ones, are complex labile materials, often required at high purity and their value depends more upon retention of functionality than upon mass;
3. most biological products are poorly characterised with respect to physical properties (e.g., viscosity, diffusivity, etc.);
4. several important bioprocess unit operations are poorly understood[80].

In order to develop simulations of biochemical processes that can deal with those characteristics that are not directly amenable to algebraic manipulation the concept of using expert knowledge and process heuristics came about. The approach attempts to solve problems using a computer model of expert human reasoning, reaching the same conclusions that the human expert would reach if faced with a comparable problem[81]. Whereas, the heuristic approach relies upon using an educated initial estimate of the final solution.

Using expert knowledge and process heuristics in combination with the traditional modelling techniques permits a greater degree of flexibility in the use of process simulation[80]. For example, the use of a purely mathematically driven bioprocess flowsheet simulator would pose some problems when it came to mass and energy balances for particular unit operations for the reasons mentioned above.

However, by being able to introduce expert knowledge that has been gained through experience about the characteristic behaviour of products and unit operations, solutions to particular problems can be readily achieved. In the case of production planning and scheduling applications, the use of process heuristics has a number of advantages.

The aim of production planning and scheduling applications is to assist in the decision making concerning the timing and sequencing of products or their intermediates onto specific equipment items. The overall aim here is to optimise the flow of products and the utilisation of equipment and resources to meet some pre-defined performance measure. The performance measure can be economically oriented, such as the minimisation of total production cost or process related like the maximisation of equipment utilisation. It has been shown that attempting to generate a solution for even relatively simple processes with a single product can be explosively combinatorial because of the number of discrete decisions that must be made[49, 55, 82].

A solution based upon a conventional analytical approach can be achieved, but with processing time penalties depending upon the size of the problem. In an attempt to reduce the complexity of the problem expert knowledge and heuristics are applied to yield near optimal solutions with reduced convergence times.

1.9 Current Trends in Simulation: Object Orientation

It is now widely accepted that automation and simulation through the use of computers is the inevitable way of improving manufacturing productivity to remain competitive[83].

Fortran has traditionally been used to develop simulation models of chemicals manufacturing processes. Fortran presents a robust and powerful programming environment. However, as is the case with high level programming languages, they require a considerable degree of expertise to use. Consequently, simulation development becomes the domain of the few. This can lead to a dichotomy in beliefs as to how the real process works: simulation developers have one understanding based upon idealised representations of the process, while those directly concerned with managing and operating the real processes have another based upon real practicalities and operational procedures. The ideal situation is to use process engineers and operators to build and maintain simulation models using software programming environments for the non-computer expert. To facilitate such requirements, current interest has focused upon the use of the object-oriented (OO) paradigm in simulation development. The relative merits of object orientation with regards to process simulation are discussed further in Chapter III.

CHAPTER II

Analytical Process Model

2.0 Introduction

The aim of this chapter is to present an analytical description of an existing monoclonal antibody manufacturing facility. Process design and scheduling for optimal economic return from a semi-continuous, multi-stage mammalian cell culture process is considered via two case studies. Firstly, heuristics are identified for the optimisation of the production phase duration, assuming a linear accumulation of monoclonal antibody. These heuristics are used to gauge the validity of the commonly accepted process design assumption that the harvest titre be maximised irrespective of the fermenter configuration. Secondly, these heuristics are further tested against performance predictions for non-linear cell culture data.

In the first instance a description of the process is presented, showing a generalised process flowsheet arrangement. This is followed by a characterisation of the feasible production patterns or fermenter schedules that are employed. A representative cost model is developed, which provides a basis around which process heuristics are evaluated.

2.1 Biopharmaceuticals Manufacturing Procedures: Process Description

Generic procedures for primary biopharmaceuticals manufacture by mammalian cell culture are as follows. Cells are first revived from a frozen cell bank vial into a few millilitres of growth medium and then cultivated through a train of progressively larger fermentation vessels until culture in a Principal Growth Vessel (PGV) is achieved. This vessel may be up to 15,000 litre scale and the entire process of cell culture from revival to its inoculation may take in excess of 35-50 days, dependent upon the growth characteristics

of the particular cell line[84, 85]. The industry has subsequently employed two patterns of product harvesting. Some companies harvest a single batch of product from the cells of each such revival using either a conventional batch or extended, fed-batch culture process. The PGV usually serves as the production vessel and, after harvesting, the vessel is cleaned and sterilised to receive cells from the next revival. This approach aims to overcome regulatory concerns over the relationship between product integrity and duration of cells in culture since each product harvest is taken from cells of similar age. This argument, and the manufacturing productivity of the approach, rely upon precise scheduling to ensure that cells from successive revivals reach the production vessel upon demand since production delays are otherwise incurred. In theory, this schedule might be precisely determined from cell growth studies. In practice, it is difficult to predict due to the imprecise nature of cell growth, the errors associated with measuring cell densities and the random, unintended occurrence of vessel contamination.

Specifically for this analytical model a different pattern of production is addressed. In this case cell culture is sustained in the PGV through successive "draw-and-fill" cycles (this vessel is then commonly called a Solera tank by analogy with traditional fermentation processes). With this approach a certain proportion of cells are (ideally) fed from the PGV to one of a number of production vessels at the end of a fixed duration growth cycle. Both vessels are then fully charged with sterile-filtered growth media; the PGV to commence a new growth cycle and the production vessel to allow the formation of product by either a batch or extended culture process. At the end of the production cycle, which would be of several days duration, the media from the production vessel is harvested in order that the product can be extracted and purified through downstream processing. The production vessel is then cleaned and sterilised to be ready to receive a fresh inoculum from the PGV at the completion of the PGV cycle. This approach is more economical in the utilisation of capital assets, operating personnel, media and other cell culture consumables, gives higher plant productivities and can be more precisely scheduled than the single harvest per revival method.

However, rigorous validation studies are required to demonstrate batch-to-batch product integrity and to establish the maximum permitted duration of cells in culture post revival[86]. All the cited figures and nomenclature used for this chapter may be found in Appendix A. Figure 2.1 presents a generalised diagram of all of the main stages associated with the existing manufacturing facility. Figure 2.2 presents a more detailed process flowsheet diagram of the culture stage of the manufacturing process. Here, the train of progressively larger fermentation vessels is shown leading up to the PGV. Figure 2.3 shows the detail of the production stage of the manufacturing process.

2.1.1 Specific Process Description

This section will provide a specific description of the manufacturing process that is the subject of this thesis. Figure 2.1, 2.2 and 2.3 are still applicable in this case, however, a more detailed description of the process flow is provided.

2.1.1.1 The Nutrient Media Preparation Stage

The media preparation stage of this process consists of two blend vessels, designated MB1 and MB2. The respective media preparation capacity of these vessels are 3500 and 8000 litres.

Both of these vessels provide the main source of nutrient media for all of the culture vessels in the manufacturing process. Nutrient media prepared in these vessels passes through a filtration unit prior to delivery.

2.1.1.2 The Culture Stage

The culture stage consists of a train of four fermenter vessels of progressively larger capacity (Figure 2.2). The first vessel in this train is designated cv100 (vessel1). The second, third and fourth vessels are designated cv600 (vessel2), cv1250 (vessel3) and cv8000 (vessel4) respectively. The cv8000

vessel is often also referred to as the Solera or Principle Growth Vessel (PGV).

The cv100 vessel is initially inoculated with a pre-defined volume of seed culture. Pre-prepared nutrient media is then added to the cv100. The volume of nutrient media added is such that the cell density of the initial inoculum is diluted down to a pre-defined value. This cell density is termed the minimum seeding density (χ_{min}). The cv100 vessel is then ready to enter into a fermentation cycle of approximately two days in duration. At the end of this cycle a further addition of nutrient media is made such that the χ_{min} condition is once again achieved. Again the cv100 vessel enters into a two day cycle. Each successive cycle is know as a sub-culture. Sub-culturing is continued until there is sufficient culture volume in the cv100 to transfer a fraction of this volume to the next vessel in line. In this case the next vessel in line would be cv600.

As with the cv100 vessel, cv600 goes through the same stages of nutrient media additions and dilution to the χ_{min} condition for each sub-culture. Further, the cv100 vessel is maintained for several sub-cultures as a back-up to the cv600 vessel. Once there is sufficient culture volume and biomass in cv600 a fraction of this culture volume is then transferred to the next vessel in line. The next vessel in line to the cv600 vessel is the cv1250 vessel. The cv1250 and cv600 vessels then go through a number of sub-culturing stages. The operation of the cv100 vessel is generally ceased by the time the cv1250 is in operation. The cv600 vessel now acts as a backup to cv1250.

The steady transfer of active culture from one vessel to the next eventually results in the seeding of the PGV with active culture. The cv1250 vessel then acts as a backup vessel to the PGV until the PGV reaches its maximum operating volume. Once the PGV achieves its maximum volume, it then acts as the main source of active culture for each of the production vessels in the production stage. The PGV sub-culture cycles durations are for any given campaign fixed at either two or three days. At the end of each two or three

day cycle a fraction of the volume is transferred to only one of the production vessels. The PGV and the selected production vessel are then charged with fresh nutrient media and enter into a fermentation cycle. The sequence of transfers from the PGV to the production vessels is pv1d, pv2d, pv1d and so on until the campaign is ended.

2.1.1.3 The Production Stage

The production stage is composed of two production fermenter vessels. These vessels are named pv1d and pv2d (Figure 2.3). Both vessels are identical in design.

As stated in section 2.1.1.2 active culture is transferred first to pv1d and then pv2d. The temporal separation between these two transfers being equal to the sub-culture duration of the PGV (two or three days). The production vessel durations are also variable depending upon the operating strategy. That is, when the process is operated in batch mode the production vessels operate in either three or four day cycles. However, when the process is operated in fed-batch mode the production vessel cycle is seven days with three additions of nutrient media over this period. These additions occurring on day 2, 4 and 6 of the fed-batch production cycle.

At the end of each production cycle (batch or fed-batch) the entire contents of the production vessel is passed on for final product recovery. The vessel is then cleaned and sterilised ready for the next transfer of culture from the PGV.

Fed-batch mode production campaigns differ additionally from the process already described in that the cv1250 vessel is not used as culture vessel. Instead it is employed as a nutrient media storage vessel. Media from this vessel is used to supply the production vessel media demands only. Media is not prepared in the cv1250 it is only stored for later distribution. This media is

prepared, as stated above, in the media preparation stage and delivered to the cv1250.

2.2 Process Synthesis

Fixed costs accrue uniformly in time for facility depreciation, maintenance and labour whilst variable costs arise from the use of utilities and the purchase of media, filters and other materials which are largely batch related. The PGV cycle time is determined by biological factors since it must be of the correct duration to ensure a reliable supply of active and viable cells at sufficient density to inoculate the production vessels. The duration of the production cycle can be chosen from a range provided that it is sufficiently long to ensure an adequate harvest titre whilst not too long that cell viability falls to some low level when the accumulation of culture contaminants (cell debris, host cell proteins, DNA and enzymes) would pose an intolerable burden on downstream processing. In practice, plant operability considerations demand that vessel inoculation and turnround be accomplished on a regular basis and therefore production and PGV cycles are normally selected to be an integer number of days to avoid irregular shift patterns. In financial terms, longer production cycle times incur higher fixed costs per batch but, provided product titres increase steadily up to the point of harvest, then these might be mitigated by the higher productivity for fixed variable costs. The duration of production vessel and PGV cycles must be carefully balanced for a given tank configuration. If a production vessel is not available to receive cells at the end of a PGV cycle, then the PGV contents must be disposed of to a biological kill system since delays in transferring cells would lead to loss of culture activity. Such disposals waste costly cell culture materials and incur decontamination costs.

Two classes of optimal process synthesis are evident. For processes which are to be transferred to a new manufacturing facility the choice of production phase duration is largely unconstrained as the number of PGV and production vessels can be chosen to optimise productivity (except insofar as

the capital costs of facility construction might impinge upon the product business case which is not considered here). More commonly, processes are developed for transfer to existing facilities when optimal process synthesis is constrained by the available tank configuration since only a discrete set of production phase durations precisely match the seeding pattern from PGV.

As the time allocated for drug development projects is driven largely by speed-to-market then decisions must be made on the duration of the production vessel cell culture cycle at an early stage of development. Various financial and practical considerations must then be weighed on the basis of meagre laboratory data.

In the following sections, simple heuristics will be established that can guide in such decisions. A particular heuristic example that is tested is the commonly adopted heuristic that the production phase duration should be such that the harvest titre and cell viability are maximised irrespective of the particular tank configuration.

2.3 Characterisation of Production Patterns

To illustrate the arguments thus far, the tank configuration shown in Figure 2.3 is considered throughout. With this configuration a single PGV feeds cells to either of two equal volume production vessels or to the drain for decontamination and disposal in the biological kill system. PGV contamination is not considered here and production vessels are assumed to feed either to a centrifuge for harvesting or to a kill system if contaminated. Typical production schedules for this configuration are shown schematically in Figure 2.4 where transfers between the vessels, to drain or to harvesting are represented by links between nodes on a time continuous network[87] . Figure 2.4 illustrates how a two day Solera cycle (hereafter denoted S2) would be operated with either a three or a four day production cycle (P3 or P4) allowing a minimum of one day for harvesting and turnaround of each

production vessel. Hereafter, this plant configuration and these production patterns will be denoted by the notation {IS2,2P3} and {IS2,2P4} respectively. Figure 2.4 shows that different durations of the production cycle affect the efficiency of utilisation of PGV batches. For the pattern {IS2,2P3} the availability of fresh cell inocula from the PGV vessel precisely matches the demand from production vessels. Every PGV batch is used to seed a production vessel and no PGV batch is disposed of directly to the kill system. Further, no production vessel lies idle for longer than the single day required for cleandown and sterilisation. This production pattern results in a balanced utilisation of fermenters.

By contrast, for the production pattern {IS2,2P4} every third PGV batch must be disposed to the kill system since no production vessel is available for seeding when it reaches that stage in its cycle of operation (see Figure 2.4). When a production vessel does become available to receive a fresh inoculum no PGV is at the correct phase of its culture cycle to provide cells: production vessels then lie unproductive for two days between batches. This production pattern results in poor balancing of fermenter utilisation and the asynchronous flow of materials is more costly for media and sterilising filters (for lost PGV batches) and incurs fixed cost penalties whilst production vessels lie idle. These costs may only be offset if sufficient extra product is produced by the longer production cycle.

Figure 2.4 also shows that each production pattern is characterised by a certain time duration after which the sequence of operations is repeated. This repeat duration (denoted R) is 4 days for {IS2,2P3} and 6 days for {IS2,2P4}. During the repeat period the number of harvests (denoted h) is equal to the number of production vessels. If the number of non-productive disposals of the PGV to the kill system within a repeat period is denoted d and the duration of a PGV cycle is S (days), then:

$$R = (h + d).S \qquad (2.1)$$

Since any pattern must contain at least one production cycle and one vessel turnround cycle, then:

$$R - P \geq T \qquad (2.2)$$

where P is the duration of the production phase, in days and T is the minimum time required to clean and sterilise a production tank ready for the start of a new batch. Operationally, production vessel turnround can be reliably completed in one day, so from (2.1) and (2.2):

$$(h + d).S - P \geq 1 \qquad (2.3)$$

Both h and d must be integers, as are S and P for practical purposes, so constraint (2.3) can be used to calculate the values of h and d corresponding to the minimum value of R for a given production pattern. To illustrate, Table 2.2 gives values of h, d and R for a range of typical production patterns. From Table 2.2 , it is noted that balanced lines have R-P=1.

2.4 The Linear Performance Problem

Consider the case where the product of cell culture accumulates linearly with time during the production cycle. This case is considered for analytical expediency to identify putative design heuristics. It is in general artificial since product accumulation from mammalian cell culture is commonly a non-linear process but has an approximate validity for actively growing cultures over relatively short time spans. For example, we have found that a linear pattern approximates the increase in titre of a humanised monoclonal antibody (MAb-A) from a recombinant CHO cell culture in a production vessel over the period from 2 to 5 days (see Figure 2.5). In this particular example the batch culture was harvested at 5 days since cell viability had by then fallen below the target minimum level. Had the culture proceeded longer, then the antibody accumulation rate would eventually have fallen. In this case the linear

assumption would incorrectly bias in favour of longer production cycles in the analysis that follows.

Let the net total financial gain which accrues from the manufacture of purified bulk product from harvests during the repeat period, R, be denoted Y, the internal value of purified bulk product which derives from each harvest be α, the variable cost of primary processing and purification for material from each production batch harvest be β, the variable cost of media, materials and decontamination which are incurred by disposal of a PGV batch to the kill system be δ, and the fixed cost per day of facility operation be τ. To a reasonable approximation for modelling:

$$Y = (\alpha - \beta).h - \delta.d - \tau.R \qquad (2.4)$$

Assuming that the installed secondary processing capacity is sufficient to process whatever quantity of antibody that can be obtained from a production harvest with equal cost efficiency and yield, then α is proportional to the harvest titre, which is assumed here to be a linear function of the production cycle duration, i.e.:

$$\alpha = \alpha_0.P \qquad (2.5)$$

where α_0 is the daily rate of accumulation of internal (company) value of purified bulk product which derives from a single production batch harvest.

To a good approximation each production batch, and the subsequent purification processes, use the same quantity of media, filters and other raw materials, and incur the same costs for quality assurance; then, the parameters β, δ and τ are not a function of harvest titre but are fixed per batch. Also, since repeat periods are of varying duration for different production patterns then the appropriate objective function for performance optimisation is the daily average financial gain Y' (= Y/R):

$$Y' = \alpha_0.h.P' - \beta.h/R - \delta.d' - \tau$$

where P'=P/R and d'=d/R. From (2.1), 1/R = I/(h.S) - d'/h, and so:

$$Y' = \alpha_0.h.P' + (\beta - \delta).d' - (\tau + \beta/S) \qquad (2.6)$$

Y' is to be optimised subject to the inequality constraint (2.2), which can be rewritten:

$$1-P' - I/(h.S) + d'/h \geq 0 \qquad (2.7)$$

In addition, the duration of the production batch is limited by the requirement to maintain cell viability above some critical value. If this duration is denoted ϕ, then the further constraint is imposed, $P \leq \phi$, or:

$$P' \leq \phi.(I/(h.S)-d'/h) \qquad (2.8)$$

In addition, $P' \geq 0$ and $d' \geq 0$. Inferences about optimal production patterns for a range of production situations can be drawn from this theory.

2.4.1 Case Study; MAb-A Production

Consider first the optimisation of the objective function Y' in the continuous (P', d') space for the manufacture of MAb-A with a 2 day PGV cycle and the production pattern {1S2,2P_}. The fixed and variable costs of production for purified monoclonal antibodies in a licensed manufacturing facility have been evaluated, from which the typical values of the economic parameters required to optimise Y' are given in Table 2.1 (scaled by a factor of 10^4 for brevity). For these parameters equation (2.6) can be written:

$$Y' = 40.P' + 3.d' - 5 \qquad (2.9)$$

If for MAb-A production the maximum permissible value of ϕ is 4 (see Figure 2.5), then (2.9) must be optimised subject to the constraints (2.7) and (2.8), i.e.

$$0.75 - P' + 0.5.d' \geq 0 \qquad (2.10)$$

$$1 - 2.d' - P' \geq 0 \qquad (2.11)$$

$$P' \geq 0 \qquad (2.12)$$

$$d' \geq 0 \qquad (2.13)$$

Constraints (2.10) - (2.13) define a feasible region in the continuous (P',d') space in which Y' is to be optimised (shown as the region ABCD in Figure 2.6). Consider the variation of Y' along the lines AB and AC for the economic parameters of Table 2.1 :

$$\left(\frac{dY'}{dd'}\right)_{AB} = 23, \text{ and } \left(\frac{dY'}{dd'}\right)_{AC} = -77$$

As d' increases Y' rises moderately along AB and then falls more rapidly along AC. The optimum value of Y' therefore occurs at the vertex A (i.e. when P'= 0.8, d'= 0.1, Y'= 27.3) at the boundary of the feasible region.

	Linear Model Economic Parameters	Non-linear Model Economic Parameters
α_o	200000 (£/Batch/day)	20000(£/m^3/g/Batch)
β	40000 (£/Batch)	40000 (£/Batch)
τ	30000 (£/day)	30000 (£/day)
δ	10000(£)	10000 (£)

Table 2.1 Values of Economic Parameters employed for the optimisation of the linear MAb-A production and the non-linear production of MAb-B.

Now consider the operability constraint that production and PGV cycles be an integer number of days to avoid irregular shift patterns. Comparison of the

optimal values of P', d', with the practical alternative production schedules (i.e. with integer values of P to retain regular shift patterns) shows:

{IS2, 2P3}; P'= 0.75, d'= 0, Y'= 25.0

{IS2, 2P4}; P'= 0.67, d'= 0.17, Y'= 22.3

For the economic parameters employed, and with the PGV and production vessel configuration considered here, the 3 day production phase process provides a higher economic return than the 4 day process even though it yields a lower harvest titre.

The generally accepted heuristic that the production phase should be selected as long as possible, irrespective of tank configuration, fails for this linear model. Notwithstanding, this assumption is the basis for much current research in mammalian cell culture to extend the period over which product can accumulate whilst maintaining the cell viability and productivity above some critical target level.

Various fed-batch and extended culture techniques have been proposed to increase ϕ. Their effect is to rotate the boundary line AC (Figure 2.6) clockwise about the point C, moving the vertex A to the right along the line AB. As Y' increases monotonically with both P' and d', then its optimal value also increases with increase in ϕ. From (2.7) and (2.8) vertex A has coordinates $(1/S-h/(\phi+1), \phi/(\phi+1))$. As ϕ tends to infinity, then Y' tends to the the maximum limiting value of $(\alpha_0.h - \delta/S - \tau)$ or 36.5 for the parameters of Table 2.1 . This time continuous trend is depicted in Figure 2.7 for the production pattern {1S2, 2P_} (and in Figure 2.8 for the pattern {1S3, 2P_}).

The practical constraint of regular shift operation, which restricts P and d to integer values, does not allow the trajectory of allowable operation patterns to follow that of Y' for continuous variation of P in Figure 2.7 , since not all integer values of $P= \phi$ lie at the vertex A. Production patterns for {IS_, 2P_} and their corresponding values of Y' and (R-P) up to a 9 day production

phase are shown in Table 2.2 . Only those practical operating patterns for which R-P=1 lie at the vertex A and display local optima in Y' fall on the optimal trajectory in Figure 2.7 . Other patterns with R-P>1 lie off the optimal trajectory of Y' and in certain cases Y' falls as ϕ is increased. This effect is shown more dramatically for the pattern {1S3, 2P_} (see Figure 2.8) where increasing ϕ from 5 to 6 or even 7 days (say) results in a less economic process. Only when ϕ is increased from 5 to 8 days is the financial return improved.

	P	d	R	P'	d'	Y'	(R-P)
{1S2,2P }	3	0	4	0.75	0	25.0	1
	4	1	6	0.67	0.17	22.3	2
	5	1	6	0.83	0.17	28.7	1
	6	2	8	0.75	0.25	25.8	2
	7	2	8	0.88	0.25	30.8	1
	8	3	10	0.8	0.3	27.9	2
	9	3	10	0.9	0.3	31.9	1
{1S3,2P }	3	0	6	0.5	0	15.7	3
	4	0	6	0.67	0	22.3	2
	5	0	6	0.83	0	29.8	1
	6	1	9	0.67	0.11	22.7	3
	7	1	9	0.78	0.11	27.1	2
	8	1	9	0.89	.0.11	31.6	1
	9	2	12	0.75	0.17	26.2	3

Table 2.2 Typical values of linear performance parameters for the various durations of production phase for the class of schedules {1S2, 2P_} and {1S3, 2P_}, employing the economic parameters of Table 2.1 .

2.4.2 Influence of Variable Cost Changes on Optimal Production Pattern

The sensitivity of the optimal product pattern to decreases in product value or cell productivity (ie. a fall in the value α_0) or increase in raw materials costs (increase in β) can be determined. The effect of each of these changes can first be visualised in Figure 2.6 . The optimal production pattern remains close to the vertex A until the gradient of contours of constant Y' becomes

parallel to one of the bounding lines AB or AC. At this point the optimal pattern switches to either vertex B or C respectively. In algebraic terms, from (2.6):

$$\left(\frac{dP'}{dd'}\right)_{Y'} = \frac{\delta - \beta}{\alpha_0 h} \qquad (2.14)$$

Along AB:

$$\left(\frac{dP'}{dd'}\right) = \frac{1}{h} \qquad \text{from (2.7)}$$

and, along AC:

$$\left(\frac{dP'}{dd'}\right) = \frac{-\phi}{h} \qquad \text{from (2.8).}$$

So the optimal value changes when $\alpha_0 = \delta - \beta$ or when $\alpha_0 = (\beta - \delta)/\phi$.

Consider changes in optimal production pattern resulting from variations in α_0 for values of the other economic parameters in Table 2.1 . This could only occur if α_0= -3 or 0.75. The former is impractical as $\alpha_0$cannot take a negative value. In practice, α_0 might change to 0.75 but such an extreme fall in product value is unrealistic; were it to occur then the optimal value of (P',d') would move to the vertex C on the line P'=0 corresponding to a negative value of Y'. As such a production process is unrealistic then for all sensible values of product value the {IS2, 2P3} production pattern is optimal (provided raw materials costs are unaltered).

Consider next changes in the parameters β or δ. The optimal production pattern would switch to the vertex C (i.e. P'=0) if, for the current product value, the value of the objective coefficient $(\beta - \delta)$ increased to a value of 80. Again, such a dramatic increase in raw materials costs is wholly unrealistic and would result in an uneconomic process.

2.4.3 Robustness of Optimal Production Pattern to Batch Failures

Consider now the robustness of the optimal process pattern to batch failures due to contamination of a production vessel. First, suppose that upon failure the run is aborted, the production vessel contents disposed of to the biological kill system and the vessel cleaned and sterilised ready for commencement of the next scheduled production batch (which might not necessarily correspond to the earliest possible next production batch).

Say on average 1 in every κ such batches fail irrespective of the duration of the production cycle (a reasonable assumption since failures commonly result from operator error during tank turnround rather than from chance events during vessel operation). During κ repeat periods κh production batches are run of which $h(\kappa-1)$ succeed and h fail. Only the successful batches are processed to purified bulk product incurring cost β per batch and generating financial return. The failed batches incur costs for media, filters and other consumables that are approximately equal to those incurred in the disposal of PGV contents (i.e. δ). In this case, the modified average daily economic gain ($Y''=Y/\kappa R$) is given by:

$$Y'' = \alpha_0 h\left(\frac{\kappa-1}{\kappa}\right)P' + (\beta-\delta)\left(\frac{\kappa-1}{\kappa}\right)d' - \left(\tau + \frac{\beta}{S}\left(\frac{\kappa-1}{\kappa}\right) + \frac{\delta}{S\kappa}\right) \qquad (2.15)$$

An identical equation to (2.14) can be derived from (2.15) so the choice of optimal production pattern is unaffected by κ or the failure of certain production batches. That is, the selection of {IS2, 2P3} in preference to {IS2, 2P4} is robust to production batch failures. However, profitability falls. For example, the limiting value of Y'' as ϕ tends to infinity is now $(\alpha_0.h.(\kappa-1)/\kappa - \delta/S - \tau)$. For the parameters of Table 2.1 and the production pattern {1S2,2P3} the maximum value of Y'' is 34.5 for a 5% batch failure rate (c.f. 36.5 for no batch failures).

Next, suppose that upon failure the production batch is aborted and the vessel made ready for immediate inoculation from the first available PGV batch, which might otherwise have been disposed of to effluent. This strategy permits rapid restart of production and avoids vessel downtime but is only an option for those production patterns with redundant Solera cycles (i.e. the option would not exist for {1S2,2P3} but might exist for {1S2,2P9} with a 60% probability). This adjustment of production schedules does not affect the selection of optimal pattern but corresponds to a phase shift in the schedule. The optimal pattern is therefore robust.

2.5 The Non-Linear Performance Problem; MAb-B Production

The analytical predictions of the linear model are now compared with the behaviour of a non-linear antibody production. Figure 2.9 shows the accumulation of a second monoclonal antibody (MAb-B) together with the viable and non viable cell densities during extended culture of a recombinant NS0 cell line. In these extended cultures the period over which the cell viability was maintained above the critical level was prolonged to permit the accumulation of higher antibody titres.

Equation (2.4) remains valid whilst equations (2.5) and (2.6) become:

$$\alpha = \alpha_0 f(P) \qquad (2.16)$$

$$Y' = \alpha_0 . f(P)/S + (\beta - \delta - \alpha_o f(P))d' - (\tau + \beta/S) \qquad (2.17)$$

Equation (2.17) is to be optimised subject to the constraints (2.2), (2.3), (2.12) and (2.13). For ease of calculation and to interpolate the data, the antibody production data in Figure 2.9 is correlated by a fourth order polynomial. This provides an analytical form for the concentration of MAb-B as a function of culture duration, i.e. $f(P) = \sum_{i=0,4} a_i P^i$. (see Table 2.3)

a_4	a_3	a_2	a_1	a_0
-0.167	4.689	-38.238	141.18	-108.56

Table 2.3 Values of the fourth-order polynomial coefficients that describe the temporal variation of MAb-B antibody titre during fed-batch culture.

Using the same β, δ and τ values as those in Table 2.1 (but now with $\alpha_0 = 2$ to correct units but maintain the same specific value of antibody as that implicit in Table 2.1 for MAb-A), consider the set of production patterns {1S2,2P_}, for which acceptable sets of P, d and R are the same as those given in Table 2.2 , together with the set {1S3, 2P_} for which sets of P,d and R are given in Table 2.5. The corresponding values of Y' for P(=ϕ) from 3 to 17 days (Table 2.4 and Table 2.5) are plotted in Figure 2.10.

P	d	R	d'	R-P	f(P)	Y'
3	0	4	0	1	83.93	78.93
4	1	6	0.17	2	101.75	63.33
5	1	6	0.17	1	123.27	77.68
6	2	8	0.25	2	158.60	75.05
7	2	8	0.25	1	213.88	102.69
8	3	10	0.30	2	291.20	112.38
9	3	10	0.30	1	388.69	151.38
10	4	12	0.33	2	500.44	162.81
11	4	12	0.33	1	616.56	201.52
12	5	14	0.36	2	723.16	202.69
13	5	14	0.36	1	802.32	225.30
14	6	16	0.38	2	832.14	204.16
15	6	16	0.38	1	786.72	192.80
16	7	18	0.39	2	636.13	137.53
17	7	18	0.39	1	346.47	73.16

Table 2.4 Values of non-linear performance parameters for various durations of production phase for the class of schedules {1S2, 2P_} employing the economic parameters of Table 2.1

Consider the {1S2,2P_} pattern shown in the above table, first without restriction on ϕ. There is initially a marked drop in Y' as P is increased from 3 or 5 days (for which R-P=1) to 4 or 6 days (R-P=2), which parallels that for linear production. Thereafter the average daily financial gain increases steadily to a sharp maximum at P=13 days, which corresponds to the point at which the antibody titre plateaus (Figure 2.9). This behaviour reasonably

supports the accepted heuristic regarding the choice of production phase length. However, peak viable cell density occurs at 8 days and cell viability falls rapidly below an acceptable level immediately thereafter at 9 days. If constraint (2.3) is applied with ϕ= 8 days then harvesting must occur well short of the maximum Y'.

Next, consider the {1S3, 2P_} pattern shown in Table 2.5. The trend in Y' is now more erratic with local optima in Y' at 5, 8 and 11 days demonstrating the general failure of the accepted heuristic. Indeed, were harvesting to be conducted at day 9 (for which R-P=3) when the constraint on ϕ is imposed, then an inferior economic return would result than harvesting at day 8 (R-P=1).

P	d	R	d'	R-P	f(P)	Y'
3	0	6	0	3	83.93	51.62
4	0	6	0	2	101.75	6.50
5	0	6	0	1	123.27	77.84
6	1	9	0.11	3	158.60	66.49
7	1	9	0.11	2	213.88	91.06
8	1	9	0.11	1	291.20	125.42
9	2	12	0.17	3	388.69	125.73
10	2	12	0.17	2	500.44	162.98
11	2	12	0.17	1	616.56	201.69
12	3	15	0.20	3	723.16	189.11
13	3	15	0.20	2	802.32	210.22
14	3	15	0.20	1	832.14	218.17
15	4	18	0.22	3	786.72	171.16
16	4	18	0.22	2	636.13	137.70
17	4	18	0.22	1	346.47	73.33

Table 2.5 Values of non-linear performance parameters for various durations of production phase for the class of schedules {1S3, 2P_} employing the economic parameters of Table 2.1

2.6 Summary

The concept of line balancing has been introduced and shown to discriminate amongst the economic merits of different operating strategies for semi-continuous, multi-tank cell culture processes. Two different patterns of product accumulation were considered. For linear antibody production with a single-day production vessel turnround capability (T=1) the case studies

support the fermenter scheduling heuristic that P be selected as long as possible subject to the condition that R-P=T and $P \leq \phi$. This heuristic results in a balanced utilisation of fermenter vessels for which no PGV batch is unduly discarded to effluent and no production vessel lies idle after turnround for want of fresh cell inocula. Further, such a balanced production schedule yields the optimal financial gain irrespective of reasonable changes in variable and fixed cost structure or frequency of production batch failures. This result contradicts the generally assumed heuristic that harvest titre should be maximised irrespective of fermenter train balancing and arises from the operational requirement for fixed shift patterns. The failure of this assumption arises from the fact that the incremental financial benefit which results by prolonging production vessel cultures (at the expense of balancing) is offset by the additional costs of discarded PGV batches or unproductive use of capital assets.

The optimum production schedules that have been identified have all been specific to the case T=1. Whilst production vessel turnaround in a single day seems reasonable, and has been shown to be commonly employed, longer turnaround times have a profound effect upon the optimum production pattern. For example, production schedules similar to those in Figure 2.4 show that the production pattern {1S2, 2P3}, for which R-P=1, is no longer balanced for the case T=2. In this case one in every three PGV batches must be disposed to drain since no production vessel is ready to receive a fresh inoculum. Also, after turnaround each production vessel lies idle for one day to await the completion of the next PGV batch. The pattern {1S2,2P4}, for which R-P=T, similarly results in every third PGV batch being disposed to drain but this loss is better justified by a longer production phase, yielding a higher harvest titre, and no idle period for production vessels. The economic return for {1S2, 2P4} is thus preferable to that from {1S2, 2P3} if T=2. This general behaviour of production vessel utilisation is repeated for the schedules {1S2,2P5} and {1S2,2P6}, though now in each case half the PGV batches are disposed to drain. Qualitatively, the heuristic R-P=T for optimum production schedules still applies.

For non-linear antibody production the superiority of balanced fermenter train operation is less marked. The case study suggests that for certain tank configurations when harvesting is done during the period of peak antibody accumulation rate (due to the constraint on ϕ) some marginal financial benefit might be had by operating an unbalanced, asynchronous fermenter train. Whether this marginal benefit is justified by operability considerations requires detailed analysis to evaluate the efficiency of labour utilisation, cost of raw materials inventory and the risk of PGV or production batch failures for different operating patterns.

It likely that the unbalanced line operation would be rejected because the additional media make-up and tank operations, the increased load to effluent treatment and raw materials inventory required for unduly discarded PGV batches, together with the increased risk of PGV and production batch failure due to operator handling errors, would not justify the marginal gain.

CHAPTER III

Simulation Model Design Concept

3.0 Introduction

In the preceding chapter a simplified analytical model for a biopharmaceutical manufacturing facility was presented. This chapter will describe a simulation model that enables a more detailed study of the same manufacturing facility. The initial focus of the discussion will be to introduce the primary and secondary software tools used to construct the process simulation model. The relevant figures cited for this chapter are located in Appendix B.

3.1 The Model Development Environment

A number of tools exist that may be used in simulation development. These range from procedural programming languages, such as Fortran, C and Pascal to application development environments and expert system shells. For example, in the United States in 1972, there were over 170 programming languages available[88].

In order to make any use of a procedural programming language the developer must have a thorough understanding and experience of the language of choice. This requirement generally leads to the choice of language being based more upon familiarity rather than suitability for the task. Secondly, a programming approach will inherently be time consuming, mainly due to the volume of coding that would need to be written. This coding would need to encapsulate both the static and dynamic relationships between the relevant variables and parameters. Depending upon the application of a simulation there may be a requirement to be visually interactive (for example a flight simulator).

To bring simulation technology to a wider audience with a relatively limited programming knowledge and expertise, software has been developed to

facilitate the rapid development of simulation models. Such software has presented simulation developers with graphical icons that can be customised to look and behave like any object that is part of the real system of interest. The simulation developer no longer has to concern themselves with the coding required to create the object. Any programming effort is linked solely to describing the behaviour of the objects that constituted the simulation. The G2™ software tool provides such an environment for simulation development and deployment and the following sections discuss some the features of G2™.

3.2 Introduction to the G2™ Development Environment

G2™ is an application development tool, distributed by the Gensym Corporation. G2™ provides a development environment for creating and deploying intelligent real-time applications[89]. In this case the term application refers to a simulation model of the biopharmaceutical manufacturing facility introduced in Chapter II.

Any application that is developed using G2 is called a knowledge base (kb). A single kb can be built up from a number of separate units, known as modules (when the kb is known as a modularised kb). Modularisation allows large complex models to be developed from smaller, more manageable pieces. Modularisation allows a number of model developers to work on separate areas of the same model, independently.

Models are built in G2™ upon workspaces. In effect the G2™ workspace is the software equivalent of a piece of blank paper, waiting to have information (knowledge), relating to the design and operation of a model, written on to it. For a model of a manufacturing process, knowledge can take the form of process data such as equipment operating parameters, costing data and rules defining the operation of individual equipment items and the process as a whole.

Such real process knowledge is captured and represented within the G2™ environment as graphical objects. For example, parameters and variables that are defined for a process are represented as parameter and variable objects. These objects serve to define the specific characteristics of the parameters and variables involved. In this way workspaces provide a further level of model organisation, below that of modularisation. Figure 3.1 presents a schematic of how a model created with G2™ can be structured.

It is apparent from Figure 3.1 that any object placed upon a workspace can itself have a workspace. This is particularly useful, not only for organisation of process data in the form of parameters and variables, but also for describing the operational details of a process. Considering the process described in Chapter II, it is possible to define the various stages of this process in a hierarchical format with an increasing level of structural and operational detail with each step down the hierarchy. That is, the process may be differentiated into a number of stages. Each stage is represented by an object upon a workspace. Each object in turn will have a workspace holding more objects, representing a higher level of detail than the previous workspace. In this way complex real systems can be modelled with a high level of organised detail. The hierarchical structuring for the simulation model of the process presented in Chapter II is discussed in detail later in this chapter.

The G2™ environment also supports what is known as rule based reasoning. That is, the interactions between the various objects of a simulation model are definable by sets of rules. The rules are able to draw conclusions from existing knowledge, to react to certain kinds of events, and to monitor the passage of time. The following is an example of the text of a rule:

if the level of any tank = 0

then conclude that the status of the tank is empty

In this case the **level** and **status** are both attributes of a tank that are monitored. An inference is made about the value of the **status** attribute based upon the value of the **level** attribute. The next example of a rule is only slightly more involved; however, it serves to illustrate the general simplicity of rules used to run simulation models using G2™.

for any valve

if the status of the valve is broken

then inform the operator that "The valve [the name of the valve] is broken."

The above example of a rule is known as a generic rule. That is, it will apply to any item that is named as a valve. In this case, when the **status** of any valve is determined to be broken then a message is displayed indicating the specific valve that is broken.

All rules are written in a natural language format, as is seen from the two previous examples. This format encourages a greater degree of understanding for the user and developer compared to attempting to interpret numerous pages of traditional computer programming language scripts. Another advantage of using such rules is that the transference of real process knowledge to the simulation model is more readily achieved.

Process knowledge in the form of process flow diagrams can also be captured and represented as objects upon workspaces. That is, each item of real process equipment has its object equivalent within the G2™ environment. This suggests that the G2™ development environment is already equipped with graphical (object) representations of real process equipment. However, this is not the case. G2™ allows models of any real

system to be developed and is therefore a highly flexible application development system. This flexibility is attributed to the fact that G2™ supports a programming paradigm known as Object Oriented Programming (OOP). OOP represents a major change from the procedural programming languages, such as Fortran that have traditionally been used for simulating complex processes. At this stage it is relevant to present a discussion about OOP, as the term 'object' has been mentioned frequently in the preceding section. Further, an understanding of OOP will provide a basis for understanding the current model development and for any future revision of the current simulation model.

3.2.1 Introduction to the Object Oriented Paradigm

The early generations of computer languages were designed for communicating sequences of instructions to computers. Using these languages, complex real world process modelling became a very difficult and an often inefficient undertaking.

Many of the applications that were developed using these conventional procedural languages found themselves becoming rapidly overtaken by the demands placed upon them. Subsequent upgrading was just as difficult as developing the original application.

The Object Oriented (OO) paradigm, by contrast, was invented to model complex systems and provide a rapid means of updating existing applications. OO methodology was seen as a software extension of the innate abilities of humans to understand complex systems by a process of abstraction. That is, humans handle real life problems by breaking them into more understandable and manageable pieces. Each piece of the problem emphasised specific important details. Each piece may itself be further broken up as required. In this way a piece by piece picture evolves, enabling better understanding and revealing routes to tractable solutions. So a

complex manufacturing process can, for example, be broken into a manageable hierarchy of pieces of information.

3.2.1.1 History of the Object Oriented Paradigm

Object Oriented (OO) technology is not an entirely new approach to modelling complex systems. The earliest use was in 1957, by the designers of the American 'Minute-Man' missile system.

The fore-runner to present day OO languages was SIMULA, developed in 1967. SIMULA provided a language that was ideally suited for modelling the behaviour of complex systems[90]. The 1970s saw the arrival of programming languages, such as, SMALLTALK and ADA[91, 92] . The present day derivatives of these programming languages were designed to better support the OO paradigm. Other, non-OO languages had extensions added to allow for object modelling.

Examples of such languages that support or have been extended to provide for an OO approach include LISP, Eiffel, C++ and Objective-C.

3.2.1.2 Basic Concepts of the Object Oriented Paradigm

The following provides a general outline of the object oriented paradigm. This approach is necessitated by the different range of terminology used by different object paradigm practitioners and gurus.

The term 'object' is used to represent any real world entity or concept that plays a specific role of interest (to the modeller) in a system[90, 92]. Each object has its own characteristic capabilities, qualities and individuality. By accurately representing the elements of a real process using objects, it is possible to reproduce the behaviour of the process by causing the objects to act out their roles within the model. Examples of objects include a motor car, a bottle, a person, a pump, a service, a sale or an order.

Each object in a model would have information associated with it that reflected those characteristics of interest. These characteristics, in turm, would determine how individual objects interacted with one another.

To achieve a fully OO modelling framework, there are a number of elements that must be supported in the programming language of choice. The main elements are the concepts of Identity, Inheritance, Encapsulation and Modularity[91, 93, 94].

3.2.1.2.1 Identity

Identity means that data are quantified into discrete, distinguishable entities called objects. Each object has its own inherent identity. Therefore, two objects that have the same name and other attributes, would still be distinct entities.

3.2.1.2.2 Inheritance

In OO systems, inheritance defines a relationship among object classes. In continuing to define the relevance of inheritance, it is only proper to explain object classification and its role in attaining the OO paradigm.

3.2.1.2.3 Object Classification

Object classification represents a process of grouping together objects that have attributes and behaviour in common. Each member of a class is referred to as an instance of that class. The class may be seen as a mould from which the required number of copies of the object is created. However, a single level of classification is often not sufficient to describe an object in detail.

Consider, for example, objects classified as belonging to the class Equipment. There is not a single instance of this class to be found in the real

world. Although, items of equipment do exist in the real world, they are all of specific types. Therefore, the name Equipment is a generalisation.

The class Equipment may have a number of sub-classes, which in turn may have further sub-classes. This hierarchical structure allows increasing levels of detail to be specified about the various types of equipment present. If, for example, only the one class called Equipment existed then we could still proceed to create a useful model. However, such an approach would prove to be inefficient in representing all the specificities of every equipment item. Consider a single instance of the class Equipment representing an aerated reactor vessel, then that instance would have all the necessary attributes to define the behaviour of a reactor vessel. However, this same instance would also posses all the attributes that define the behaviour of a pump. Clearly, these are two distinctly different equipment items.

A more efficient and convenient method of description is to create sub-classes of the class Equipment. The highest (superior) class will specify attributes that are common to all equipment items. These attributes will be inherited by every sub-class and the final instance of the object. It is not only attributes that can be inherited in this way. Any coding that is fixed to a particular superior class is also inherited.

Classification and consequently, inheritance, helps to keep programs shorter and more tightly organised[92]. The idea of classification is illustrated in Figure.3.2.

3.2.1.2.4 Encapsulation

A model of a complex system will inevitably consist of a large number of objects. Ideally, these objects will interact with each other in an ordered and representative manner. An OO modelling approach, however, will not allow any object to access or be accessed directly by another. Communication between objects occurs exclusively through explicit messages. As an

example, if we have an object class that represents a 'reactor vessel' in a model, this class would contain all of the information needed to be kept for each instance of the class (name, volume, pressure, temperature etc.), and would encapsulate the procedures (codes) that would update the volume, the pressure and the temperature. Therefore, it is of no concern, to other objects, how a reactor vessel updates its own data.

3.2.1.2.5 Modularity

Modularity, discussed previously, provides an organisational component to the OO paradigm. Complex models can be built up as a group of interrelated modules. One module may define the object classes and coding for a specific segment, while another defines those for another segment. In this way, an entire team of modellers are able to work independently on the same model.

It is now apparent that the basic elements (identity, inheritance, encapsulation and modularity) of OO programming and design have a high degree of synergy[91]. They are not separate or isolated elements of the OO paradigm. Instead the existence of one necessarily requires and promotes the existence of one or more of the other elements.

3.2.1.3 Summary

The OO paradigm aims to provide a framework upon which complexity can be more conveniently modelled. This is achieved by allowing the abstraction of real world systems into manageable data objects.

Each object contains all the information necessary to allow it to carry out its unique function. Therefore, the maintenance of the overall model is simplified. Updating the model requires only those objects directly affected to be modified.

Abstraction in this manner allows object libraries to be constructed. Such libraries are then able to aid in the re-use of objects for other models. The modelling of, say, manufacturing systems then becomes just a question of connecting the required objects (machines, operators, parts etc.) together. However, the OO paradigm does have a number of draw backs. Productivity improvements through re-use starts only after a significant library exists.

Creating a library requires each object to be designed and vigorously tested. An end user of this library must understand the library well, before any complex modelling can be attempted. This implies significant development time and costs.

In addition to G2™, a second software tool was used to expedite model development and analysis. This tool was called ReThink™. The ReThink™ software was itself developed using the core G2™ product.

3.3 Introduction to ReThink™

ReThink™ is a graphical object-oriented simulation tool that enables complex processes to be rapidly modelled. Dynamic process simulation models are constructed as networks of connected process activities. The ReThink™ environment provides a core set of object blocks designed to carry out a range of different, but also generally observed processing activities within business and manufacturing environments.

Examples of these object blocks include the Task Block, the Source and Sink Blocks and the Branch Block. As its name suggests, the Task Block is used to represent the main resource consuming activities of a process. Diverse examples of real tasks include taking a telephone call, or operating a piece of manufacturing equipment. Each of these tasks requires some level of resource assignment. To take a telephone call requires someone to answer the phone and also the phone itself may be considered as a resource. Operating an equipment item will consume both utility and personnel

resources. In effect, the Task Block is used to represent those value adding activities of any process.

The Source Block is used to create objects that are inputs to a process. For example, a Source Block can be used to create telephone calls or any other kind of external stimulus or impetus. The Sink Block is the counterpart to a Source Block. Sink Blocks are used to signify the end of a particular line of processing. Taking the telephone call example again, if the call leads to a sale of goods then the process continues. If however there is no sale then no further information is generated and the process ends. The Branch Block is used to represent decision making within the process. Several kinds of decision making are supported. For example, decisions can be based upon probabilities, upon values of process parameters or on the type of objects passing through a Branch Block.

The object blocks described above are just some of the processing blocks provided to develop process simulation models. A more exhaustive list and description of the processing blocks provided by the ReThink™ environment may be found in the ReThink User's Guide[95]. For the purpose of this study, the above mentioned object blocks and one other block were sufficient at this stage to develop the simulation model. This other object block is the Copy block. As the name suggests any work object arriving at this block will be duplicated and the two copies are then passed down separate paths. The specific dynamic structure of a model is then dictated by how these object blocks are connected together to form a process activity network (PAN).

The basic mechanics of any ReThink™ process model involves creating, processing and deleting objects in a process via a combination of processing blocks, such as those described earlier. For example, a model of a manufacturing process might:

- Generate raw materials
- Process those raw materials into a final product
- Deliver the final products to customers or store the final product

In such a model the raw materials and the final product are all referred to as work objects. A running model will show several work objects moving from one processing object block to the next. The work objects not only serve to illustrate the flow of material round the process but also to transfer information from one processing object block to another.

3.3.1 ReThink™ Process Modelling Paradigm

ReThink™ simulations are driven using a discrete event simulation paradigm[79, 95]. Each change in the system that occurs in real time is called a discrete event. Each event has a start and stop event. The discrete event clock advances with every new stop event. The amount by which the clock advances represents the duration of each Task Block in the process model. This concept of discrete event simulation for ReThink™ process models is illustrated by Figure 3.3 . This figure shows a simple model consisting of three blocks. Initially (1) all the blocks and the simulation clock are idle. However, once the model is started (2) the source block is first activated, marking the beginning of the first discrete event. The source block then enters into the stop event and generates a work object (3) and passes it downstream to the waiting task block. The simulation clock now advances in time. The next event (4) is marked by the activation of the task block when the work object arrives. When the task block has completed processing the work object, it then enters into the stop event and passes the work object on to the sink block (5). The simulation clock accordingly advances again. The sink block will proceed to delete the work object and the process comes to an end. The whole process has taken a simulated 30 minutes, while the task block processed the work object for 15 minutes.

3.3.1.1 Probing the Performance of the Model

Simulation models of various systems are developed for a number of reasons. However, generally speaking simulation models are used to gather process performance data for given changes to initial conditions of the process.

Process performance data can be gathered in a number ways within the ReThink™ environment. The simplest of these is by using objects called instruments, in the ReThink™ terminology. The relevant instrument is simply attached to the processing block that computes the process statistic of interest. A description of the complete range of instruments available is found in [95]. This, however, is not the only way to probe for process performance data. The relevant data can be outputted directly to a spreadsheet and further data manipulation carried out. The latter of the two methods has been used to produce the data presented in this study, although instrument probes have also been used to produce run time performance profiles.

The discussion thus far has been presented so as to provide a suitable level of understanding of the development environment used to construct a simulation model of the biopharmaceutical process described previously in Chapter II. The following discussion will focus upon that simulation model, specifically dealing with its design and the concepts behind that design approach.

3.4 An Introduction to the Design of the Simulation Model

For convenience and brevity, the monoclonal antibody manufacturing facility that will be the focus for the remaining sections of this chapter and as described in Chapter II will be referred to simply as the 'process' or the 'process of interest'.

The impetus for developing a model of the process has come from many fronts, including the research and development arena, from the

manufacturing operations and from process managers concerned generally about maintaining the cost efficiency of the process. Because of the spread of interests involved the final model developed had to be such that it could be understood and used by all those groups. Importantly, it must also be remembered that the process will also evolve over time. Therefore, there is a requirement to ensure that the simulation model can be easily updated to encompass changes as they occur. These changes must also be capable of being easily inserted into the model by those people who would use such a system, remembering that the familiarity of these individuals with computer software may well be limited to the use of the ubiquitous Microsoft applications.

3.4.1 Spreadsheet Based Process Model

The first attempt to develop some form of process model manifested itself as a spreadsheet based data model. This model, however, gave only a very simple and static picture of the process. No costing or kinetic models of cell growth and product accumulation were included. The main calculations within the spreadsheet centred on prior knowledge of what the initial and final cell densities should be for each active vessel.

Since the cell density elements were fixed and always known, the main use of the spreadsheet became to predict the total media usage and waste volume levels over a campaign. Figure 3.4 shows the complete spreadsheet model and may be found in Appendix B. Campaign time advances down the spreadsheet, while various process operations between vessel 1 and 2 are shown horizontally across the spreadsheet. The process parameters presented are updated mainly in the horizontal direction.

3.5 An Introduction to the ReThink™ Process Model

The spreadsheet based data model was not sufficient to provide users with a useful simulation tool enabling various process scenarios to be investigated.

A greater degree of flexibility in application and in useful information generated is required. The spreadsheet model was also unable to reflect both the material and information flows of the process in any meaningful manner and thereby restrict any interpretation of optimal process operation and subsequent re-design.

A new dynamic process simulator ranging in its application from being able to evaluate variable operational scenarios to providing a safe training environment for process operators is needed. Clearly, the spreadsheet model presented previously is limited in its provisions for such requirements. Further, the generally static nature of spreadsheets prevents such dynamic requirements.

Key to building a useful process simulator is to ensure a high degree of understanding of basics behind how that simulator works. In the first instance, this is usually achieved by creating a graphical environment with which the user is familiar. Contemporary process simulators, particularly those used within the chemical process industries have a common 'front end' process representation format. That is, the graphical environment with which the user generally interacts. This format is that of the process flow diagram (PFD). The user is then presented with an interface with which he/she can easily relate to.

The PFD approach to process representation is taken with the ReThink™ process model. However, the conventional PFD is extended not only to represent the process flow from one unit operation to another, but also to represent the underlying individual process activities associated with the unit operations. That is, each unit operation is seen only as an integrated representation of a number of definable and discrete activities. These activities themselves also form a dynamic PFD.

The inference from this is that the process model has a hierarchical construction. Indeed, this is how the description of the ReThink™ process

model will be structured. The simulation model will be presented and described in a top down manner.

3.5.1 Overall Process Schematic Diagram

In Chapter II a description of the real manufacturing process is given. This description presents the process, initially, as a four stage process. The four stages being Media Preparation, the Culture Stage, the Production Stage and the product Recovery Stage. The same representation of the process is also used in the process simulation model. Figure 3.5 shows a screen shot of the four stages. Each stage is represented by an object block and the blocks are connected by paths as they are in the generalised diagram in Chapter II.

A number of other objects are also shown in the above figure, of which the most important is the one labelled as the Scenario Controller. This object is the graphical representation of the discrete event simulation engine that is used by ReThink to run simulation models. The other objects are either folder objects used for organising other objects or they are action buttons. The action buttons are used to determine at the start of any simulation run what particular process operating mode is to be used, that is, either batch or fed-batch modes.

Each of the four process stages shown have a further level of process detail. The detail of the Media preparation stage is shown in Figure 3.6, while the detail for the Culture and Production stages are shown in Figure 3.7 and 3.8 respectively. The detail for the Recovery Stage is not presented primarily because this stage of the process was not originally intended to be studied.

In Figure 3.6 the single Media Preparation Stage object block has now been differentiated to reveal a greater level of process detail. The individual media blend vessels are now apparent along with the media filter unit and the relevant path connections (piping).

The detail revealed for the Culture and Production stages can be regarded as the equivalent of a conventional PFD. All the relevant unit operations are shown (culture and production vessels) and the connection paths (piping) between them. The process of differentiation, however, still continues. Each vessel represented in the above three figures has a further level of detail associated with it. This structural detail is specific to a particular process stage, that is to say that a vessel in the Media Preparation Stage will have a detail different from a vessel in the Culture or Production stages.

3.5.1.1 Process Differentiation: Culture & Production Vessels

The operation of batch biopharmaceutical processes may be considered as consisting of a number of definable activities. Each of these activities will have a determinable start and end time. For example, take the preparation of nutrient media which has a number of steps involved. These steps will generally be ordered in some way and each step will take a given amount of time to complete. Similarly, other process activities may be viewed in the same way. Using this knowledge, that is the knowledge of the basic process defining activities, the operation of a biopharmaceutical process can then be described by a number of discrete activities occurring over time. Once such activities have been defined, they can be connected together rather like a PFD. The sum of the parts (activities) then constitutes a given unit operation. Each activity block that is defined contains an attribute table which lists all of the relevant parameters or variables and their initial values. As the simulation model runs these attributes are updated and are available for charting.

As stated previously, the mechanics of any ReThink™ model involves the creation, processing and deletion of objects via a combination of processing blocks. These objects were termed work objects. For the process of interest in this case, the work objects represent discrete quantities of **Media** and active **Culture**. A running model in this case will show instances of **Media** and **Culture** moving from one processing block to another.

For the operation of a culture vessel in this process, four basic process activities have been defined as constituting the functioning of a culture vessel. These are listed as follows:

1. Vessel **Filling**
2. Cell **Growth/Product Formation**
3. Culture **Transfer**
4. Vessel **Cleaning**

This set of core activities represents a generic level of activity for our process. Each of these activities when connected together in a logical manner form the underlying detail of a specific unit operation and of the process as a whole. This activity based approach is analogous to a project network technique known as Activity-on-Node (AoN)[96].

Essentially, the AoN technique involves representing the system of interest by a series of paths/arrows and nodes (boxes or circles). This representation effectively displays the logic of the system, i.e. '*This activity must follow or precede that activity*'. Figure 3.9 shows the AoN representation for the operation of a culture vessel. Following the arrow from the far left we firstly encounter the vessel **Filling** activity. After vessel **Filling** is complete, the arrows then lead to the cell **Growth** activity. The growth activity is then followed by the culture **Transfer** activity, after which the next possible activity is that of vessel **Cleaning**. Whether the vessel is cleaned or not is determined by operational requirements. If, for example, the reactor vessel is to be maintained as a backup to the next reactor vessel then there would no need to clean it. The arrow marked culture recycle would be followed. If, however, it is decided that the culture vessel is not to used as a backup or is contaminated then the **Cleaning** activity would be initiated. Figure 3.9 effectively is a flowsheet depicting the underlying operational logic for the operation of any culture vessel.

Since the production vessel in this process can be viewed as scaled up versions of the culture vessels then it possible to use the same activity sequence to define the operational logic of each production vessel. Figure 3.9 shows only a generalised schematic of the underlying process activity logic associated with the operation of any culture or production vessel. The actual ReThink™ representation of the same diagram is more complicated as shown in Figure 3.10. The general logic structure shown by Figure 3.9 is still clearly visible in Figure 3.10. however, there are a number of additional blocks and flow paths evident.

Considering the **Growth** activity first, it can be seen that there is another object block attached to the top of the **Growth** activity block. This object block is called a Source block and has been described previously. The role of the Source block in this instance is to feed externally derived data to the **Growth** activity regarding the next vessel in line, the volume of culture that is to be transferred to that vessel, and the new required total volume of the vessel for which the **Growth** is an activity. Externally derived data means that data is supplied to the ReThink™ model from an external data file. This data is encapsulated within a work object generated by the Source block. The Source block is activated only once in every PGV cycle, the trigger for this being an update in the total vessel volume which is monitored within the **Filling** activity.

Between the **Filling** and **Growth** activities there exists a circular node; similarly, between the **Growth** and the **Transfer** activities. These nodes are used to represent the sampling process. That is, prior to starting a vessel a sample is usually taken to check for contamination and to measure biomass levels. This corresponds to the first node, between the **Filling** and **Growth** activities. Prior to transfer of culture another sample is again taken for the same reasons. If during any of these sampling stages a contamination is detected then vessel cleaning and sterilisation is initiated. This accounts for the second output path from each node leading to the vessel **Cleaning** activity. A third sampling node is located after the **Transfer** activity; however,

this represents a redundant feature that is bypassed during the running of the process model.

There are also a number of object blocks displaying the '?' symbol. As has been stated previously, the ReThink™ environment provides a number of processing blocks that help to define different processing activities. In this case the '?' represents a Branch block. The Branch block enables decision making based upon a number of different criteria. The role of the Branch block is in effect analogous to the IF, THEN, ELSE statements ubiquitously found in programming languages.

The role of the first Branch block, downstream of the **Transfer** activity, is to decide which output path will be taken by any work object on its input path. This decision is based upon the value of a specific attribute of the work object on the input path of the Branch block. In this instance the Branch block looks at an attribute named *sub-status*. The *sub-status* attribute shows whether the current culture or production vessel is to be maintained as a back-up to the next vessel, that is whether this vessel will be sub-cultured or not. This attribute can hold only true or false values. Therefore, if the *sub-status* value is false then the vessel for which the Branch block is an activity will no longer be operated and must be cleaned and sterilised. The work object is passed along the output path on the right of the Branch block. If the *sub-status* value is true then the work object is passed along the output path at the bottom of the Branch block.

There are number of other such Branch blocks located in close proximity to the Branch block just described. The role of these blocks is much the same in that they decide whether the current vessel is to be maintained in operation or stopped, cleaned and sterilised. In each case, the Branch blocks look at different attribute values.

Below the main process network diagram a three stage process network diagram is shown. This network represents another activity that is an integral

part to the whole process. This activity is the **Steaming** of transfer lines. Since such an activity is applicable to all items of process equipment, it has been represented as an independent activity not requiring any of the other activities.

A further level of process activity is resolved, for the **Filling**, **Transfer** and **Cleaning** activities, with each of these activities in turn being made up of a further level of detail. The orange colouration (Figure 3.10) of the above mentioned object blocks signifies that they have a further level of detail associated with them.

3.5.1.1.1 Filling Activity Detail

The process of filling a culture or production vessel has three 'sub-activities'. When such a vessel is first active, culture material from the previous vessel must be transferred to it. Nutrient media is then prepared and directed to this vessel. Hence, the vessel is charged with culture and then nutrient media and so two 'sub-activities', *fill-with-media* and *fill-with-culture*, are defined. The third 'sub-activity' is strictly speaking not a an activity as they have been defined so far. This 'sub-activity' effectively represents the calculation of the total vessel volume. That is, the sum of the media volume and the culture volume from the *fill-with-media* and *fill-with-culture* activities respectively. This third 'sub-activity' is termed the *post-fill-update*.

The reason for increasing the degree of abstraction of the **Filling** activity was so that more precise resource allocation was possible. Although differentiation between the 'sub-activities' could have been achieved programmatically using only the **Filling** activity, such an approach would not be as easily understood as a graphical representation of the process logic. Figure 3.11 illustrates the abstraction of the **Filling** activity into its relevant 'sub-activities'.

3.5.1.1.2 Transfer Activity Detail

The **Transfer** activity detail is primarily intended to reduce the programmatic coding effort needed to differentiate between when a transfer will or will not occur and hence to define when an operator resource is required. In this respect two 'sub-activities are defined for the **Transfer** activity. These are labelled *Transfer-Solera-A* and *Transfer-Solera-B*. The former of these two activity blocks is used when the volume of culture to be transferred is greater than zero, while the latter is concerned with a zero culture transfer (no transfer of culture). Figure 3.12 shows the ReThink™ process activity network diagram for the **Transfer** activity. It can be seen that there is a Source Block connected to *Transfer-Solera-A*.

The role of this Source Block is to ensure that a transfer of culture material from the vessel for which the Transfer is an activity cannot take place while the destination vessel is still active. Once the destination vessel becomes available the Source Block generates a work object, which is passed to *Transfer-Solera-A*. Only now can the *Transfer-Solera-A* complete its processing and start the transfer of culture material. In effect having two input paths to an activity means that the activity cannot start its processing until both paths are active. The same transfer control logic is applied to all the culture vessels.

Although this particular **Transfer** activity may seem trivial to codify, in an attempt to make the design and functioning of the simulation model easily understood, it was decided that a graphical representation of the decision making process would be best. However, if every decision making element or calculation involved in the simulation is defined using a graphical representation then the model becomes too complicated. Hence, where necessary graphical representations have been replaced with rules and programming.

Consider, for example, the cell **Growth** activity. This activity represents that part of the real process when cell density is increased and product is formed.

Therefore there is a requirement for some means of predicting the cell density at the end of this growth period. Similarly, we need to be able to predict the concentration levels of any product that might be formed. These requirements are achieved through the execution of cell growth and product formation models. Such models are best expressed through a conventional programmatic approach rather than attempting to represent them in a logical graphical format. A similar argument is applied to the cost modelling of the process. The cell growth and cost models used for this simulation model are discussed in greater detail later in this chapter.

3.5.1.1.3 Cleaning Activity Detail

As stated previously, the vessel **Cleaning** activity also contains a further level of detail. The creation of a further level of detail for vessel **Cleaning** is necessitated because the act of cleaning a vessel is broken into a number of stages. Of particular prominence is the need to empty, where necessary, the contents of the vessel to be cleaned. The relevant ReThink™ schematic for the **Cleaning** activity is illustrated in Figure 3.13.

The process activity network that defines the **Cleaning** operation consists of two basic 'sub-activities'. These are vessel **Emptying** and vessel **Cleaning** activities. There is also a single Branch, Source and Sink block. The Branch block in this case distinguishes between whether the vessel needs to be emptied before it is cleaned or whether cleaning can start immediately. Situations requiring such a decision occur, for example, when a culture or production vessel transfers its entire contents to the next vessel or the next stage of product processing respectively. The Sink block serves to delete the work object generated and so mark the end of the **Cleaning** activity. The Source block connected to the *empty-vessel* sub-activity provides a means of operating any culture vessel as a media holding and distribution vessel. When a vessel is used as a media holding vessel, as is the case for the CV1250 vessel during fed-batch operation, the normal process activity network is not followed. In fact only the vessel **Filling** activity is employed. As

media is distributed from this holding vessel to the production vessels it is not likely that the entire volume of stored media will be used. A residual amount is normally left. This must be disposed to waste as it is not sufficient to meet the demands of any of the production vessels. This is where the Source block initiates the removal of the residual media.

The preceding discussion has presented an explanation of the ReThinK™ process model structure as far as the operation of the culture or production vessels is concerned. The following section will deal with the design of the media preparation stage and specifically the media blend vessels.

3.5.1.2 Process Differentiation: Media Blend Vessels

The media preparation stage of the manufacturing process consists of two media blend vessels of different volume capacity. The functioning of media blend vessels will be different to that of the culture and production stage vessels previously described, although there will some similarity in the types of activities involved. For example, there is still a requirement for a vessel **Filling** activity and also a **Transfer** activity.

A new set of activities is defined for the media preparation stage and specifically for the media blend vessels that constitute this stage of the manufacturing process. As before, with the culture and production vessels, each activity that is defined also defines an attribute table listing relevant parameters and their initial values. The relevant media preparation stage process activities are listed as follows:

1. Vessel **Filling**
2. Media **Mixing**
3. Media **Transfer**

The process activity network representation for the media blend vessels is shown in Figure 3.14. As the only difference between these two vessels is in

their maximum capacity, the final process activity network diagrams are identical for the two media blend vessels.

3.5.1.2.1 Media Filling Activity Detail

Unlike the vessel **Filling** activity of the culture and production vessels, the media **Filling** activity dose not have a further level of detail, since in this case the concern is only to fill with media. The relevant information as to how much media and the final destination of that media is supplied in one of two ways.

The first of these methods is a manual approach, whereby, the user enters the relevant nutrient media requirements for each vessel via an operator interface. This information is passed to the media **Filling** activity of the relevant media blend vessel. The user then initiates the media preparation sequence.

The second approach is more automated, where the decisions as to how much media and where to send it are made by the simulation model itself. This approach is implemented via a sequence of rules that basically mimic the sequence of decisions that would have to be made if the manual approach were employed. For the current simulation model, this automated approach is only partially implemented due to time constraints. The set of rules that exist to enable the automatic sequencing of media have been tested and validated for combinations of only the culture stage vessels. Although the rule exists for combinations of the culture and production vessels, sufficient time was not available to test and validate these rules. Hence, they are deactivated. This limitation means that once the production vessels are operating the user must revert to a manual approach to the preparation of nutrient media.

3.5.1.2.2 Media Mixing Activity Detail

As with the media **Filling** activity, no further level of detail is associated with the media **Mixing** activity. This activity is used purely to represent a necessary hold-up in the process that equates to the approximate time taken to fully mix the nutrient media.

3.5.1.2.3 Media Transfer Activity Detail

The media **Transfer** activity, like that of culture vessel **Transfer** activity, also hides a further level of abstraction. However, in this case the **Transfer** activity is required to distinguish between multiple media transfers to culture and production vessels.

To explain, consider the situation where a multiple request for media is made by two of the culture stage vessels, one being the PGV, and by one of the production vessels. Assuming that the total media demand can be met by one of the media blend vessels then the total required volume of nutrient media is prepared. However, once prepared the nutrient media must be broken into batches representing the volumes required by each of the culture vessels and the production vessel. Once this segregation has occurred the nutrient media can be directed to the right vessels. Therefore, the media **Transfer** activity is composed of six media **Transfer** 'sub-activities'. Each one of these 'sub-activities is responsible for directing media to the one vessel specifically. In reality, the multiple transfer of media is handled simply by feeding the required media volume to one vessel and then starting on the next vessel. However, codification of such a process can again become complicated. So a diagrammatic approach is used to expedite understanding. The process activity network for the media **Transfer** activity is shown in Figure 3.15.

The discussion thus far has presented the structural design of the simulation model. The emerging picture has been that of a hierarchical approach to designing the simulation model, where the basic idea has been to decide

what the core processing activities are that constitute the process as whole. In effect the process has been modelled from the bottom up. This approach serves to identify and even isolate specific activities to which resources can be more accurately assigned. Further, such a structural approach eliminates a great deal of the coding effort initially required to build the simulation and later to aid in the understanding of how the model works and how it can be adapted.

Now that the concepts behind the simulation model structure have been presented it is only correct that there be some discussion of the programmatic structure supporting the simulation. Discussion of the written program structure provides a basis for understanding how the kinetic and cost models were implemented.

3.6 Simulation Model Program Structure

The program or code structure of the simulation model is broadly speaking organised into two types. There are those elements of coding that aid in the control of the model structure and dynamics. The second grouping of codes define the relationships or formulas that allow the numerous process statistics to evolve over the duration of a simulation run. Where the term codes or coding is used, the actual reference is to the use of rules, similar to those shown previously. The first of the rule types that will be considered are characterised as design rules, while the second group are process rules.

3.6.1 Introduction to the Simulation Model Design Rules

This section will present a number of the rules that have been created to control the simulation model. It is not intended that all such rules will be presented. Moreover this section will provide a general guide to the type and nature of the Design Rules.

The structure of this simulation model has, as has been explained, a hierarchical format. In order for information to be exchanged between the different levels of detail for a given object a relationship has to be created. For example, considering any one of the culture vessel shown in Figure 3.7 , it is known that there exists at least two further levels of detail below this object. Ultimately, a user of this simulation model would not be concerned with these lower levels. However, it is in these levels that all the processing related to the operation of the vessel is carried out. Hence, the relevant process parameters (attributes) are calculated and updated in these levels. Since the user will interact only with the level showing the vessels themselves, the process data must be made available to the vessel object as well. An example of a rule that achieves the initial creation of a relationship is shown in Figure 3.16.

This rule basically looks to see when a particular attribute (the counter) of an activity (referred to as an operations) receives a value. When a value is received a relationship (an-operation-detail-for) is created between that activity and the vessel object superior to the object. The end of this rule invokes another set of rules called relation-to-tank-rules that basically updates process parameters. Examples of such parameters are the total vessel volume, which is calculated by the *post-fill-update* **Filling** 'sub-activity' or the vessel productivity which is calculated by the **Growth** activity. Similarly worded rules also exist to relate the data generated in the media processing details to the media blend vessels.

An example of an attribute table for a culture vessel is shown in Figure 3.17. Each of the attributes listed in the table shown in Figure 3.17 is calculated by different processing activities at differing levels of process detail. The attribute table of any of the vessels in the media preparation, culture and production stages represents a common point from where data is made available to the simulation user.

The rule shown in Figure 3.16 is a generic rule. That is, it is invoked by any activity (operations) when the counter attribute of that activity (operations) receives a value. Therefore, this rule need only be written once and will control every activity (operations) that has a counter attribute.

A further example of a Design Rule is shown in Figure 3.18. This rule is used to trigger the Source block that is attached to the **Growth** activity of every culture and production vessel (see Figure 3.10).

Again, this rule is generic in nature as indicated by the term 'any' in the rule body. In effect this rule waits for a particular event (increment in the counter value) to act as a trigger for another event (the activation of a relevant Source block).

The two rules thus far represent just a small fraction of the total volume of such rules developed to aid in the control and information handling of the simulation model. They demonstrate the relative ease with which control of simulation models can be achieved with limited programming knowledge. The rules themselves are self explanatory because of the natural language format. Importantly, here the rules that have been described are also generic in a process independent sense: irrespective of what the product of the process is, these rules will still apply. They form the backbone of a generic simulation model.

3.6.2 Introduction to the Simulation Model Process Rules

In the light of the closing statement of the preceding section regarding the generic nature of the design rules in terms of application to other processes, the Process Rules then define the specific relationships between various attributes for this manufacturing process. To explain further, consider the determination of final cell densities and product concentrations at the end of a growth or production cycle for any vessel. For this process, such variables will be defined by specific cell growth and product formation models. Also,

different operating configurations will have differing operational requirements. For example, during fed-batch operation of the process, the volume of nutrient media fed to the production vessels is based upon a percentage of the current volume of culture in the vessel. Different processes may have other criteria for determination of nutrient media feed volumes. This simulation model, for example, is able to distinguish between two operating configurations at the touch of a button, these configurations being batch and fed-batch.

The examples of Process Rules used in the process simulation model will be focused upon the kinetic (cell growth and product formation) models and how they are specifically incorporated using a rule based programming paradigm. At this stage the opportunity to describe the development of the kinetic and cost models is also taken.

3.7 Kinetic Model Development & Rule Based Implementation

The overriding interest of this work is in plant scheduling, process benchmarking and debottling. For this purpose cell growth has been modelled relatively simply for batch and fed-batch operating configuration using two different empirical methods. The first method, as applied to batch operation, uses a simple exponential growth equation. The second, applied to the fed-batch configuration, employs linear approximations. Both models have been developed using actual process data.

3.7.1 Batch Process Cell Growth Model

The typical cell growth profiles for batch processes exhibit 4 characteristic phases[25, 97]. The first of these phases is known as the lag phase. During this phase there is generally very little cell growth seen and then a design objective would be to minimise this period. The length of this phase depends on the previous history of the inoculum - increasing the similarity between the previous inoculum stage and the culture medium conditions will reduce the

time of the lag phase[98]. After the lag phase a period of rapid growth begins, during which the cell numbers increase exponentially with time. This is referred to as the exponential growth phase. Within a closed vessel, cell growth cannot continue indefinitely. Following the growth phase a stationary phase is entered, where the cell population achieves its maximum size. The stationary phase is then followed by a phase of exponential cell death.

This cell growth behaviour pattern is typically presented with respect to the growth of microbial cell cultures. However, the same general principles behind the origin of such a profile is still applicable to animal cell cultures, the biggest difference between microbial and animal cell processes being seen in the magnitude of the cell numbers achieved. Microbial cell processes produce significantly higher cell numbers than animal cell based processes.

For both the batch and fed-batch processes, the culture stage vessels are all operated in a batch draw and fill mode: when one vessel has completed its processing duration a pre-determined volume of the vessel contents is drawn off and transferred to the next vessel in line. Both vessels are then fed with nutrient media and processing may start once again. This operation is repeated until all the culture vessels, that are to be used, are active. The transfer of culture from one vessel to the next is initiated such that the cells to be transferred are still in their growth phase. This serves to eliminate or reduce the lag phase experienced by the transferred culture.

Since the active cultures are maintained in the exponential growth phase it was only necessary to model this phase of cell growth. During this phase it is assumed that growth is not limited by nutrient concentrations and therefore, it is not necessary to account for nutrient depletion. Cell death is also not factored into this model as this phenomena only becomes significant during the death phase.

Using real process data (4162W94 campaign data) it was possible to generate average cell density profile for each vessel in the culture stage of

the process. This average profile was based upon the assumption that each cycle or sub-culture of a given vessel will produce approximately similar growth profiles. Therefore, each sub-culture profile can be used to generate an average profile for that vessel. Also, since the culture stage of the process consists of vessels all operating in a similar cyclic pattern of periods of growth followed by dilution back to (approximately) the initial cell density, then merging the average profiles for each vessel provides an average growth profile representative of the whole culture stage. Figure 3.19 shows a plot of the cell density verses the elapsed sub-culture time obtained for four culture stage vessels over their first sub-cultures. Similar profiles are presented in Figure 3.20 and Figure 3.21 showing the cell density profile for the same four vessels over the second and third sub-cultures respectively.

The above three figures represent growth profiles for the first revival of the cell line used. The following three figures present, again, the same profiles but this time for the second revival of the same cell line. Figure 3.22 shows the profile for the first sub-culture, while Figure 3.23 and Figure 3.24 show the second and third sub-culture profiles respectively.

The three profiles showing the cell growth profiles for the first revival are merged to produce averaged cell growth profiles for each of the three sub-cultures. The same is done for cell growth data from the second revival. The resultant averaged cell growth profiles are presented in Figure 3.25 and Figure 3.26 for the first and second revivals respectively.

For each of the sub-cultures presented in Figure 3.25 and Figure 3.26, the data was found to be modelled by a simple exponential correlation. The correlating exponential curves are also shown on Figure 3.25 and Figure 3.26.

These correlation curves are generally described by the following equation

$$\chi_t = \chi_0 \exp[\mu t] \qquad (3.1)$$

where χ_t is the cell density at time t (hrs), χ_0 is the initial cell density and μ is the specific growth rate (hr^{-1}).

Using a trend line fitting tool, equations for each curve were generated and presented in Table 3.1.

Revival No.	Sub-Culture	χ_0 (10^6/ml)	μ (hr^{-1})
1	1	0.32	0.57
	2	0.26	0.59
	3	0.37	0.90
2	1	0.37	0.42
	2	0.35	0.45
	3	0.32	0.54

Table 3.1 Summary table showing the values of χ_0 and μ for the exponential correlation curves.

Using the values from Table 3.1, the average values for χ_0 and μ were determined. Therefore, equation 3.1 is now completely represented as

$$\chi_t = \chi_0 \exp[\mu t] \qquad (3.2)$$

where χ_0= 0.33x10^6/ml and μ = 0.58hr^{-1}. The real process value of the minimum cell density was set at 0.35x10^6/ml. The value of χ_0 is seen to be approximately equal to this value. This observation provides a high degree of confidence in the correlation provided by equation 3.2.

Equation 3.2 now provides a means of predicting the cell density for any of the culture vessels. Also, the same correlation is applicable to the production vessels working in batch mode.

3.7.1.1 Batch Process Cell Density & Product Correlation

The average cell densities achieved by the same culture stage vessels at comparable elapsed time intervals were correlated against the average product concentrations achieved at approximately similar time intervals for the same vessels. The results of this correlation are presented in Figure 3.27. Figure 3.27 shows a plot of the average cell density achieved over a number of sub-cultures plotted against the average product concentration achieved over the same number of sub-cultures.

The above correlation basically provides the equivalent of look-up table. So, for a given final cell density achieved over any sub-culture the expected final product concentration can be estimated. The equation of the best fit line is as follows

$$[\text{MAb}] = mX_{av} - c \qquad (3.3)$$

Where [MAb] is final product concentration (units of products/ litre of culture), m = 109.8, c = 53.201 and X_{av} is cell density achieved at the end of a sub-culture ($x10^6$/ml).

Attempting to develop a product formation model along the lines of those commonly presented in the relevant literature for animal cell systems, involving multiple substrate utilisation models and cell death kinetics[25,99], would have required extensive laboratory and plant scale experimentation and this was not the focus of this work. In order to carry out these lab scale experiments, a considerable degree of training and familiarisation with sampling and assay techniques would have been needed. Since the aim here is to provide a representative production simulation where trends rather than absolutes are more important, the development of an all inclusive cell growth and production model is not necessary.

3.7.2 Fed-Batch Process Cell Growth Model

The cell growth for the fed-batch process has been modelled using a linear approximation method. Figure 3.28 shows the viable cell growth profile for two different ages of the same cell line, and also the average of the these two profiles.

The viable cell density profile shows only a small variation between the two culture ages. The particular culture ages used (25 and 166 days) present the widest possible range of culture ages for which real process data was made available. The agreement between the two profiles suggests that, despite the culture age difference, the behaviour of cells used for a fed-batch process will be approximately similar. The average profile generated can be used to predict viable cell concentrations. The linear approximations to the viable cell density is developed via a segmented approach. That is, a separate linear correlation is derived for each two day step of the average viable cell density profile shown in Figure 3.28. Table 3.2 shows the derived linear equation for each of the 2 day time steps.

Time Range (days)	Viable Cells Linear Approximation
0-2 (incl.)	y=0.265x+0.36
2-4 (incl.)	y=0.4275x+0.035
4-6 (incl.)	y=1.2475x-3.245
6-8 (incl.)	y=0.7925x-0.515
8-10 (incl.)	y=-1.4225x+17.205

Table 3.2 Summary table showing the linear equation for each two day step in the average viable cell density profile of Figure 3.28. (y = viable cell density and x = the elapsed time).

3.7.2.1 Fed-Batch Process Cell Density & Product Correlation

As with the correlation between viable cell density and product concentration for batch operation a similar approach is taken for the fed-batch correlation.

The correlation in the fed-batch case is given by an exponential relationship between viable cell density and final product concentration.

The data used to generate the fed-batch correlation originates from actual samples taken from the production vessels while they were operated under a fed-batch regime. Four sets of correlating equations were generated corresponding to four revivals of the same culture. The general form of the correlating relationship in each case is given as follows

$$Y = Ae^{bX} \qquad (3.4)$$

where Y is the final product (antibody) concentration (units/L), X is the final viable cell density ($x10^6$/ml) and A and b are constants. Table 3.3 shows the derived values for A and b from each of the four correlations.

Revival No	A	b	R^2
1	18.22	0.76	0.92
2	36.01	0.65	0.99
3	48.51	0.46	0.92
4	40.91	0.40	0.97

Table 3.3 Summary table showing the values of the constants A and b for each fed-batch exponential correlating equation between viable cell density and final product (antibody) concentration for four revivals. The correlation coefficients shown indicate a high positive degree of correlation.

The average values for A and b are hence calculated and A = 35.91 and b = 0.57.

3.7.3 Statistical Cell Growth Variation Model

The cell growth and product formation models described above provide a consistent means of predicting the kinetic behaviour of the real manufacturing process. The results from one simulation run to the next will be identical. However, for the real process and other batch processes generally, such a

situation never occurs. A number of reasons are cited to account for this variability, ranging from variability in the supply and quality of the raw materials used, the variation in the physiological state of the cell inocula from batch to batch to the idiosyncrasies of individual operator[49, 54]. Therefore, no two production runs will be identical in terms of cell growth and product formation profiles. This is evident from considering the growth profiles presented for the first and second revivals of the process operating under a batch production paradigm (Figure 3.25 & Figure 3.26).

To account for this variation, a growth variation or fluctuation model has also been included in this process simulation model. The aim in this case is not to reduce the variation in data but to incorporate this variation so as to provide a more realistic set of output values. The variation model has been implemented independently of either of the batch or fed-batch kinetic models. Consequently, the ability to introduce variations is completely under the control of the simulation user. So, simulations may be run with or without the variations.

From the data used to derive the cell growth models for the batch and fed-batch operating configuration, the average deviation in the average viable cell density profiles were determined. The two values, for batch and fed-batch operating configurations are shown in Table 3.4.

Batch: Average Deviation	Fed-Batch: Average Deviation
0.17 x10^6 cells/ml	0.60 x10^6 cells/ml

Table 3.4 Summary of the average deviations from the average viable cell density profiles for batch and fed-batch operating configurations.

These two values provide an estimate for the maximum perturbation in the final viable cell density that may reasonably be expected for any batch of active culture material in any of the culture and production vessels.

The final average cell density is then determined according to the following equation

$$x_{t(\text{var})} = x_t + (\beta\omega) \qquad (3.5)$$

where $x_{t(\text{var})}$ is the final average viable cell density (x10^6/ml) for any culture or production vessel subject to a statistical variation, x_t is the average final viable cell density (x10^6/ml) determined from either of the batch or fed-batch correlating equations, β is a real number generated in the range ($-1.0 \leq \beta \leq 1.0$) by a random number function and ω is the value of the average deviation for the specific operating configuration shown in Table 3.4.

3.7.4 Rule Based Implementation of Kinetic Models

The intention of this section will be to provide a general understanding of how specific rules (Process Rules), using the kinetic models developed above as an example, are coded into this simulation model. Figure 3.29 below shows an extract from a section model coding. As with the Design Rules presented earlier the extract below has the same language format. This section of rule coding basically checks to see whether the statistical variation model is switched on and, if so, that it then calculates the viable cell density using the batch operation exponential correlation equation and then sends this value to another set of rules which randomly alters this value to mimic real batch to batch variation. This is analogous to making a subroutine call with languages like Fortran and basic; however, the subroutine is not held within the same body of coding. If the variation model is not switched on, then only the final viable cell density is calculated.

```
begin
{
 Using a simple exponential growth model the cell growth profile is estimated. Where t1
   is in hours.
}
        if the current value of kinetic-uncertainty-model is true then
           begin
              conclude that the final-cell-density of task = the xmin of
                task*exp(mu*t1);
              call batch-cell-density-variation-generator (task)
           end else
        if the current value of kinetic-uncertainty-model is false then
           conclude that the final-cell-density of task = the xmin of task * exp(mu
             * t1);
     end
```

Figure 3.29 An extract of the simulation model coding that calculates viable cell density. The specific section shown calculates the viable cell density for batch operation.

Figure 3.30 shows another extract from the same section of model coding. This section deals with the implementation of the viable cell density model for fed-batch operation. As is seen, there is an initial conditional statement that checks to see whether the vessel (tank) for which this code has been invoked is operating in fed-batch mode. If this is the case, then depending upon the elapsed time value another set of rules are invoked that calculate the viable cell density, using the linear model described previously. In both Figure 3.29 and Figure 3.30 the language format used makes for better understanding of how the codes function.

```
else
    if the operating-strategy of tank is fed-batch or the operating-strategy of tank
      is fed-batch-draw-and-fill then
    begin
      if et<=2 then
        begin
          call viables-linear-approx-1(task, et);
        end
      else if et<=4 then
        begin
          call viables-linear-approx-2 (task, et);
        end
      else if et<=6 then
        begin
          call viables-linear-approx-3 (task, et);
        end
      else if et<=8 then
        begin
          call viables-linear-approx-4 (task, et);
        end
      else if et<=10 then
        begin
          call viables-linear-approx-5 (task, et);
        end
      else if et >10 then inform the operator above the tank that is made-up-of
        task that " The upper boundary limit for the biomass growth model has been
        EXCEEDED!!" ;
    end
```

Figure 3.30 An extract of the simulation model coding that calculates viable cell density. The specific section shown calculates the viable cell density for fed-batch operation.

3.8 Cost Model Development

The cost model used for this simulation model is a variation of that presented in Chapter II. The model used in Chapter II calculates the net total financial gain that accrues from the manufacture of purified bulk product (antibody). The model used for the simulation model estimates the unit cost of production for a batch of product as it flows through the process. Due to the serial nature

of the production process, costs are accrued as the product advances through the process. At each vessel, raw materials and utilities are consumed and process waste is generated. The cost of the resources consumed must be added to the costs that have been already incurred. The cost of a batch of product at the end of the series of cost incurring steps must finally be inclusive of the fixed costs associated with the process as a whole.

For simulation purposes, the cost of production for a batch of final product can be reasonably estimated from

$$T_{Cp} = V_{Cp} + F_{Cp} \tag{3.6}$$

where T_{Cp} is the total cost incurred in producing a final batch of harvestable product, while V_{Cp} and F_{Cp} are the total variable costs and fixed costs, respectively, incurred for the same final harvestable batch of product. The variable and fixed cost elements are further differentiated into their component costs: that is, for this particular process simulation model V_{Cp} is defined as follows

$$V_{Cp} = \left(M_C + U_C + W_C + Cl_C + A_C\right) \tag{3.7}$$

where M_c is the total cost of preparing the nutrient media, U_C is the total cost of the available utilities used, W_C is the total cost incurred in treating process waste, Cl_C is the cost of materials used to clean out a vessel and A_C is the total cost incurred for testing the product batch as is passes through the process. Similarly, F_{Cp} is defined as follows

$$F_{Cp} = \left(L_C + O_C + Mt_C + FC_C + Eq_C\right) \tag{3.8}$$

where L_C is the total cost of operating labour incurred in generating the final product batch, O_C is the cost of process overheads, Mt_C is total cost of equipment and general process maintenance incurred, FC_C is the total fixed

charges that accrue over the duration of the process, and Eq_C is the total installed equipment cost.

The waste handling (W_C)and testing (A_C) cost elements of equation (3.7) for the process are in reality treated as fixed costs. Equations (3.7) and (3.8) then respectively become

$$V_{Cp} = \left(M_C + U_C + Cl_C\right) \tag{3.9}$$

and

$$F_{Cp} = \left(L_C + O_C + Mt_C + FC_C + W_C + A_C + Eq_C\right) \tag{3.10}$$

The variable and fixed cost elements that have been thus far listed , by no means form an exhaustive list as there are many more component costs that could be included. However, those included have been because the data is readily available or at least reasonable current estimates exist and these elements represent the more significant cost elements of this process.

The cost model is implemented such that the total cost of production over each sub-culture is estimated. The initial cost at the start of the process is steadily transferred from vessel to vessel with each successful transfer of active culture. At each of these vessels, the cost calculation is based solely upon the variable cost element (V_{Cp}). Once a batch of active culture reaches either of the production vessels, then the total cost, at the end of the production vessel duration, is calculated according to equation (3.6). A more formal expression of the cost model implementation is as follows.

If n represents either of the production vessels at the end of the manufacturing process, then (n-1) represents the vessel immediately preceding the production vessel which in this case would be the PGV. Similarly, the (n-2)th vessel would be cv1250 in batch operation and cv600 in fed-batch (remembering that the cv1250 vessel is used as a media storage vessel during fed-batch operation). N defines the set of vessels up to the production vessels, thus

$$N = \{1,....(n-1)\} \text{ vessels.}$$

Also, if S is the total number of sub-cultures associated with the Nth vessel and s_t is the sub-culture at the end of which a transfer to the next vessel in line takes place, then

$$i = \{s_0, s_1, s_2, ..s_t, ..S\} \text{ sub-cultures.}$$

For any vessel belonging to N, the total cost of production (T_{Cp}) associated with the current batch of active culture held by the Nth vessel is given as follows

$$T_{Cp_{N,S}} = \sum_{i=s_1}^{i=S} V_{Cp_{N_i}} \qquad (3.11).$$

When a transfer of a batch of active culture occurs to the next vessel in line then equation (3.11) can be written as

$$T_{Cp_{N,s_t}} = \sum_{i=s_1}^{i=s_t} V_{Cp_{N_i}} \qquad (3.12)$$

and, initially, T_{Cp} for the next vessel in line is given by

$$T_{Cp_{(N+1),s_0}} = V_{Cp_{(N+1),s_0}} + T_{Cp_{N,s_t}} \qquad (3.13).$$

As the (N+1)th vessel continues, then over subsequent sub-cultures the value of T_{Cp} that is associated with the current batch of active culture held by the (N+1)th vessel is then given by equation (3.14).

$$T_{Cp_{(N+1),S}} = \sum_{i=s_1}^{i=S} V_{Cp_N} + \left(T_{Cp_{(N+1),s_0}}\right) \qquad (3.14)$$

So far, equation (3.14) defines an estimate of the total production cost incurred for the culture stage vessels only. The estimate of total production cost for the production stage vessels is given by two different equations depending upon whether the process is in batch or fed-batch operating mode.

For batch operation there are no sub-cultures of the final production batches. While in fed-batch operating mode, a final product batch experiences four fed-batch additions of nutrient media. For batch operation T_{Cp} is calculated as follows

$$T_{Cp_n} = (V_{Cp_{(n-1),s_l}} + V_{Cp_n}) + F_{Cp} \qquad (3.15).$$

The equivalent fed-batch process estimation of T_{Cp} is given by the following

$$T_{Cp_{n\{f=4\}}} = (V_{Cp_{(n-1),s_l}} + \sum_{f=1}^{f=4} V_{Cp_n}) + F_{Cp} \qquad (3.16)$$

where f is the number of cycles corresponding to the addition of nutrient media. The final value of T_{Cp} is presented as a per unit of product cost.

Table 3.5 presents the specific values of the cost components used for this simulation model, while Table 3.6 gives a breakdown of installed equipment costs for those equipment items described in the process description of section 2.1.1.

Variable costs	Estimated value	Fixed costs	Estimated value
M_C	£1.30/litre	L_C	£80/day/person
U_C : *Power*	£0.25/kWhr	O_C	£1300/day
U_C :*Steam*	£0.20/kg	Mt_C	£200/day
U_C :*Process Air*	£0.30/m^3	FC_C	£200/day
Cl_C	£200/vessel	W_C	£8/day
		A_C	£70/day
		Eq_C	£14,616/day*

Table 3.5 Summary table of the variable and fixed cost component values used by the cost model used in this process simulation model (* see Table 3.6 for breakdown of installed equipment costs).

The values of U_C shown in the above table were determined from literature examples of similar manufacturing processes[11]. The process specific data was unfortunately not directly available to the process managers. However, the estimates that are presented in the above table were agreed by the same process managers to be reasonable.

Equipment description	Installed Equipment cost estimate (£)
cv100	108,750
cv600	250,000
cv1250	300,000
PGV	500,000
PV1D	500,000
PV2D	500,000
MB1	60,857
MB2	99,937
correction factor	2.3

Table 3.6 Table showing the breakdown of the individual equipment installed costs. A correction factor is included to account for the equipment items not included in the list (See section 2.1.1 for process flow description/diagram).

The correction factor used was derived from a cost proposal for a similar manufacturing process[100] where a more extensive listing of equipment items had been presented. Hence, this proposal presented a realistic final figure for the total installed equipment cost which can then be used to determine a correction factor.

To further clarify, the installed equipment cost (Eq_C) is not added to the final product cost of each product batch as it may appear from the equations above. In fact this cost is spread over the entire duration of a production campaign.

3.9 Summary

This chapter has presented the means of modelling a complex manufacturing process by defining process activities at their most general level. Each unit operation for this process is represented, conceptually, as a network of activities. These activities are connected together to form a process activity network diagram. This network diagram presents the basic operational logic underlying the operation of a particular unit operation. The dynamic element to the process model is provided by the creation, processing and deletion of

objects (work objects) that represent the raw materials and products of the process. These objects not only show the flow of material about the process, but also represent a means of transferring information from one activity to the next and unit operation to unit operation.

The activities that have been defined represent generic operational functions that can be applied to other similar manufacturing operations. Any requirement for further activities can generally be achieved through the extension of the existing activities. In this way new process models may be developed rapidly, initially for prototyping purposes. This is achieved through the implementation of a rule based coding structure that has a generic and specific dichotomy. The generic component relates to the general design and operation of the model while the specific rule coding deals with process specific phenomena such as cell growth, product formation and cost models.

The overall rule coding structure is distributed: unlike the traditional approaches to programming, i.e. non object oriented, where all the programming is held within a single body containing numerous subroutine calls and iterative loops, the coding is split into manageable segments. In this way related segments of coding may be better organised together. For example, segments of coding related to viable cell growth modelling can be grouped on to a single workspace. Changes, where necessary, can be carried out on only those segments requiring updating without having to examine the entire code structure. Such distribution leads to the overall coding structure being more easily understood and manipulated as the future requirements of the process model change.

Complex process phenomena models relating to, say, heat and mass transfer can be written in a natural language format by a human expert or experts. These can then be easily integrated into the overall simulation model. The human experts in this case need not have any real knowledge of the manufacturing process as a whole.

Overall, the general design of the simulation model has been such that the operational logic is clearly evident and amenable to further manipulation. This manipulation is then based upon the use of the basic process activities that have been defined or the further extension of these activities to create the desired level of detail.

CHAPTER IV

Process Benchmarking Studies

4.0 Introduction

Using the simulation model described in Chapter III, a series of simulations were carried out for different process operating configurations. In the first instance, these simulations have been directed towards obtaining representative process benchmark data. The extracted data is used to characterise the existing process and also to provide a basis for comparison between selected configurations.

The contents of the following sections of this chapter will thus focus upon the presentation and discussion of process benchmarking results for the feasible process operating configurations that have been studied.

Two process operating configurations are considered. These are the conventional batch and fed-batch operating configurations. For each of these configurations, different combinations of PGV and production vessel processing durations are evaluated.

4.1 Batch & Fed-batch Operating Configuration Benchmarks

Four feasible batch process operating configuration schedules and two feasible fed-batch schedules were evaluated. The term 'schedule' in this case refers to the particular combinations of PGV and production vessel durations used. These configurations are summarised in Table 4.1 below.

Configuration No.	PGV Duration (days)	Production Phase Duration
1	2	3
2	2	4
3	3	3
4	3	4
5	2	7
6	3	7

Table 4.1 Summary table showing the combinations of PGV and production vessel durations used. Configurations 5 & 6 relate to the fed-batch operating configurations while the remaining are related to the batch operating configuration.

To maintain consistency with the nomenclature used in Chapter II, the configuration numbers are replaced with a schedule description as shown in Table 4.2. As may be deduced, the nomenclature for configuration 1 refers to a 2 day PGV cycle (1S2) and a batch production vessel cycle of 3 days (2P3). While for configuration 2 there is still a 2 day PGV cycle (1S2) and a production vessel cycle of 4 days (2P4). The remaining two configurations follow the same pattern. (The use of (1S2) to describe the duration of the single PGV stems from the traditional name for the PGV which was Solera vessel).

Configuration No.	Schedule Description
1	1S2, 2P3
2	1S2, 2P4
3	1S3, 2P3
4	1S3, 2P4
5	1S2, 2P7
6	1S3, 2P7

Table 4.2 Summary table showing the schedule description for each of the batch & fed-batch operating configurations studied.

Each of the schedules studied have been subject to a random number based variation in final cell density, as described in Chapter III. Since there is now a statistical variation built into the process performance it was necessary to repeat each simulation run up to at least 5 times. Consequently, the results presented are average values.

4.1.1 Batch & Fed-batch Configuration Benchmark Results and Discussion

For the two configurations studied (batch and fed-batch) and the operating schedules therein the following process parameters were determined.

1. The time taken to produce a pre-determined quantity of product
2. The average production vessel productivities
3. The plant schedule productivity
4. The average cumulative product cost
5. The average cumulative volume of nutrient media used
6. The total volume of biologically active waste

Further, profiles showing the peak operator allocations over each production campaign are also presented. The majority of the following analysis and discussion is focused upon the tank configuration shown by Figure 2.3. The relevant figures cited throughout this chapter may be found in Appendix C.

4.1.1.1 Project Makespan

Figure 4.1 presents the total time taken to produce a pre-defined quantity of product for each of the batch and fed-batch schedules studied Within the production planning and scheduling disciplines, the total-time taken to complete a task or project is referred to as the project makespan. In this case the pre-defined product quantity is taken as that produced at the end of the schedule with the shortest duration, that is, the {1S2, 2P3} schedule. Moving to the left of this schedule, it is apparent that the same final product quantity is achieved more rapidly by four of the schedules, while only one schedule

takes longer to meet the product requirement. The {1S3, 2P4}, {1S2, 2P4}, {1S3, 2P7} and {1S2, 2P7} schedules have the shorter product makespans, while the {1S3, 2P3} schedule has the longer makespan. Table 4.3 summarises the makespans for each of the schedules.

Schedule	Schedule Makespan (days)
{1S2, 2P7}	25
{1S3, 2P7}	32
{1S2, 2P4}	39
{1S3, 2P4}	42
{1S3, 2P3}	55

Table 4.3 Table comparing the product makespans for each schedule. The {1S2, 2P3} schedule makespan (not shown) is used as the basis for comparison and has a makesapan of 45 days.

For optimisation purposes, if the objective is to solely minimise production time and therefore maximise the speed to market of the final product then there would be no hesitation in selecting that configuration and schedule with the least value for the makespan. In this case that would be the fed-batch configuration employing the {1S2, 2P7} schedule.

However, obtaining and maintaining the optimality of a manufacturing process is not just achieved through the consideration of a single process parameter, such as the time to completion. Although the {1S2, 2P7} schedule offers a favourable makespan when compared to the other schedule studied there are still a number of equally important process parameters and operational matters that must be considered.

To gain sufficient representative data for analysis of the process performance a campaign was decided to consist of 10 batches for both batch and fed-batch schedules. In reality a campaign can consist of significantly more batches. From these 10 batch campaign time profiles for the batch and fed-batch schedules (Figure 4.2 and Figure 4.3) it is apparent that those

schedules with the shortest PGV duration (2 days) result in shorter overall 10 product batch completion times. However, the {1S2, 2P4} schedule shows a considerably longer 10 product batch completion time than the {1S2, 2P3} schedule for only a 1 day increase in the production vessel duration. While for the same increase in production vessel duration between the {1S3, 2P3} and {1S3, 2P4} schedules the difference between the completion times is less pronounced.

Clearly, the longer the production vessel duration is, the greater the time taken to completion than with a schedule with a shorter production duration. However, the effect of the PGV duration upon the final completion times has also to be taken in account. This is best achieved by examination of the time continuous network diagrams, introduced previously in Chapter II, for each of the schedules. To re-cap, these diagrams present each production schedule as a schematic where the transfers between vessels, to drain or to product harvesting are represented by a series of connected nodes.

Firstly, consider the network diagrams for {1S2, 2P3} and {1S2, 2P4} schedules shown by Figure 4.4 and Figure 4.5 respectively. Immediately obvious is the existence of idle times in the {1S2, 2P4} schedule: that is, there exists a period of time between the end of one production batch and the beginning of another where the production vessel remains idle for 1 day. This idle time results from a temporal mismatch between the PGV and production vessel cycle times. For the PGV, this cycle time is effectively equivalent to the PGV duration, since there is no requirement to clean the PGV between growth cycles. However, the production vessel cycle time is composed of the production duration and the production vessel clean-down time. The clean-down time is set at an achievable minimum of 1 day. This immediately introduces a restriction upon the availability of the production vessel. So, when the PGV is ready to transfer active culture (inocula) the production vessel is not ready to receive it and must then wait for the next transfer from the PGV. This wait constitutes the production vessel idle time.

However, a zero idle time is observed for the {1S2, 2P3} schedule. This implies that the {1S2, 2P3} schedule is a balanced or synchronised schedule, where the seeding pattern of the PGV exactly matches the inocula demand from the production vessel. Hence, it can be inferred that the {1S2, 2P3} schedule is more efficient than the {1S2, 2P4} schedule in terms of production vessel utilisation.

The same argument developed above is applicable to the {1S3, 2P3} and {1S3, 2P4} schedules. Looking at the network diagrams for these schedules shown by Figure 4.6 and Figure 4.7, it is clear that idle times exist for both schedules. The duration of the idle time is greater for the {1S3, 2P4} schedule. Hence the time taken to complete 10 batches of product for the {1S3, 2P4} schedule is marginally longer than that for the {1S3, 2P3} schedule.

As far as the fed-batch schedules are concerned the very same argument is again applicable. The existence of idle times in the {1S3, 2P7} schedule accounts for the greater 10 batch production time than that for the {1S2, 2P7} schedule, where there is no idle time. The network diagrams for the fed-batch schedules are shown by Figure 4.8 and Figure 4.9.

4.1.1.2 Average Production Vessel Productivity

Moving now to an interpretation of the average production vessel productivity data for each operating configuration and the operating schedules therein. Figure 4.10 shows the average production vessel productivity for each of the batch schedules studied, the average production vessel productivity being defined as the average productivity over a whole campaign. Commonly, process operating strategies will be such that productivity values are maximised and therefore the {1S3, 2P4} schedule would marginally be favoured over the {1S2, 2P4} schedule. The longer the production duration the more product that can be produced and the greater the measured productivity.

The average production vessel productivities attained for the fed-batch operating schedules, shown in Figure 4.11 are seen to be approximately 1.5 times greater than the best performing batch schedule. This result is not unexpected when comparing the performance of fed-batch processes over batch processes. The commonly observed better performance of the fed-batch paradigm is in part attributed to a reduction in cell growth inhibitory effects, which can occur at high concentrations of nutrient in traditional batch processes. Additionally, fed-batch operation allows for cultures to be maintained for a longer period of time while still producing product.

From Figure 4.10 a clear pattern emerges in that those schedules with the longer production vessel durations result on average in a higher vessel productivity. This is not unexpected since an increased production duration implies a higher final product concentration and also productivity.

4.1.1.3 Plant Schedule Productivity

The discussion thus far has concentrated upon the average production vessel productivity. This measure does not directly provide a means of comparing the productivity of the different operating schedules that are being investigated, beyond supporting a common process heuristic that production vessel duration should be as long as possible. To address this issue, a plant schedule productivity performance parameter is defined. Such a measure of process performance provides a means of accounting for the idle time experienced by production vessels as a result of an asynchronous operating schedule. This measure, the plant schedule productivity, is shown in Figure 4.12 and Figure 4.13 for the batch and fed-batch operating configuration schedules respectively.

The plant schedule productivity takes into account the total vessel turn round time, i.e. the production vessel production duration, the time taken to clean the vessel and any idle time that may be incurred. Initially, looking at the

batch schedules with the 3 day production vessel durations and both fed-batch schedules, it is apparent that those schedules that incur production vessel idle times have the lower overall schedule productivity values. The inference here being that in general synchronised schedules, {1S2, 2P3} and {1S2, 2P7}, are more productive than asynchronous schedules. However, the {1S2, 2P4} and {1S3, 2P4} schedules are both asynchronous and yet produce higher plant schedule productivity values than either of the two remaining batch schedules. This is accounted for by noting that the production vessel durations for {1S2, 2P4} and {1S3, 2P4} schedules are longer and since it has been established, for the batch schedules, that productivity is proportional to production vessel duration, then a higher vessel productivity is expected. This higher value then counters the effect of idle time upon the calculation of the overall schedule productivity. The concept of synchronised and asynchronous schedules are first introduced in Chapter II.

It is noticeable that of the two competing operating configuration schedules, the fed-batch are more favourable than the batch in terms of the overall schedule productivity. This observation still holds when production vessel idle times are present.

Also of importance is the need to ensure that the vessel utilisations are maintained at optimum levels. However, two factors have a significant affect upon the vessel utilisation. These are the vessel waiting times and the need to ensure that all of the day's processing activities are carried out within an 8 hour time frame. The relevance of the latter factor is not immediately obvious and an explanation follows.

In keeping with the real process, media preparation for any particular day's requirements is started first thing in the morning (8 am). This poses the problem that the required volume of media for each vessel, and hence the total media, is only known once the vessels have come to the end of their durations. However, the vessels do not necessarily complete their growth cycles at 8 am. For operational reasons, this problem is overcome by

prematurely ending the growth cycles of the vessels at 8 am. This action results in approximately 2 to 3 hours loss of growth time. The effect of this reduction upon cell growth is minimal and therefore has only a small effect upon the final volume of nutrient media needed. This procedure is not applied to the production vessels once they are active. The production vessel are always allowed to complete their entire production durations.

Profiles for PGV and production vessel waiting times are shown in Figure 4.14 for the {1S2, 2P3} schedule. The remaining schedules (Figure 4.15, 4.16, 4.17, 4.18, 4.19) show a similar profile. It is evident from these profiles that the PGV suffers the greatest overall waiting times between successive growth cycles. The production vessels do incur unavoidable waiting times but these are somewhat smaller than those for the PGV.

The reason for the longer media waiting times experienced by the PGV rather than the production vessels is that the production vessels have priority for media preparation. This condition applies particularly when concurrent demands are made for media by other vessels and when the existing capacity of the media preparation stage is insufficient to meet the total media requirements. Therefore, when such a situation occurs, the production vessel will always be first to receive nutrient media or nutrient media preparations for the production vessel will be started without delay. Once, the production vessel has received nutrient media then preparation begins to meet the nutrient media demand of the PGV. Hence, the PGV media waiting time is further compounded by the fact that media has first to be prepared and delivered to a production vessel.

An increase in the media wait times for PGV now means that the PGV growth cycle will be completed much later on during the final day of its growth cycle. However, this situation is not allowed to occur since the processing of the PGV is stopped early on the final day of its growth cycle. Hence, the PGV will then suffer a further reduction in its overall duration cycle.

The initial waiting time that a vessel experiences results from two further limiting conditions. Firstly, a limitation in the filtration rate of the nutrient media filter and secondly a low nutrient media preparation capacity. The existing media filter filtration rate is approximately 2000 l/hr. For the culture stage of any campaign, the volume of media prepared will be small in comparison to volumes required by the PGV and the production vessels. Therefore, the filter, or more specifically, the filtration rate does not pose a significant bottleneck in the operation of the culture stage vessels. However, for the PGV and the production vessels, the volumes of nutrient media per vessel can be in excess of 4000 litres. Therefore, the waiting times are at least 2 hours per vessel to which is added the time taken to actually prepare the media. This results then in a waiting time for the production vessels of about 3 hours. If there is not sufficient capacity in the media preparation stage to prepare the PGV media concurrently with that of the production vessel then the PGV must wait for the production vessel to be serviced with media. This can add up 7 hours to the PGV wait time. Therefore, the bottleneck in this case is not just the filtration rate but the media preparation capacity itself.

The media preparation stage consists of two media blend vessels. The capacity of one (MB1) being approximately 3500 litres while the other (MB2) is 8000 litres. During the initial stages of the process when only the culture stage vessels are active, the MB1 vessel has sufficient capacity to meet the total needs of these vessels. Once the PGV comes into use, there is still sufficient capacity between both MB1 and MB2. However, once the production vessels are active, only very occasionally is the combined capacity of MB1 and MB2 enough to meet any concurrent media demands from the PGV and a production vessel.

4.1.1.4 Average Cumulative Product Cost

For the majority of manufacturing processes the performance measure that carries the most weight in terms of strategic decision making, is that of the

average cost of production or some other economic measure of process performance, such as net financial gain (profit). The average cost of production profiles are shown in Figure 4.20 and Figure 4.21 for the batch and fed-batch schedules respectively. The cost model developed to determine the overall production cost is presented in Chapter III. The overall production cost trend seen in the two profiles is as expected. The greater the average productivity associated with any batch of product the lower is the corresponding average production cost per batch. Comparing the average production vessel productivity profiles (Figure 4.10 & 4.11) with the average product cost profiles for each schedule, it emerges that those schedules with the more productive production vessel cycles yield the more cost effective processes.

For the batch schedules, shown in Figure 4.20 it is apparent that for a one day increase in the production vessel duration (3 days to 4 days) an approximate halving of the production cost results. This result conforms to expectation as higher productivity is expected for longer vessel duration and therefore a lower cost per unit of product.

4.1.1.5 Average Cumulative Media Volume Used

For pharmaceutical processes, and particularly those involving animal cell dependant processes, the cost of nutrient media is a major area of concern. Often very high costs are incurred due to the unique nutrient requirements of the animal cells used. There is often very little that can be done to minimise this cost. However, there is potential to reduce nutrient media consumption by selection of those process configurations and schedules that make the best use of the least possible media.

The profiles shown in Figure 4.22 and Figure 4.23 are the average total volumes of nutrient media consumed by the production vessels over the duration of each production campaign for the batch and fed-batch schedules respectively. Comparing the profiles using the PGV duration as a basis, it is

found that for those schedules where the longer PGV duration (3 days) exist the volume of nutrient media consumed by the production vessels is greater than in the 2 day schedules.

This observation is attributed to the fact that for longer PGV durations a higher final cell density is achieved for the PGV culture. Therefore, in order to reduce the cell density back to the initial starting cell density for the beginning of the next growth cycle, a greater volume of nutrient media is required.

Further study shows that the total volume of media consumed by the production vessels operating under the fed-batch schedules is comparable to that of the batch schedules. This implies that the yield of product from a given volume of media is significantly more for the fed-batch configuration than for the batch. Therefore, better utilisation of costly nutrient media is achieved with fed-batch operation. However, in terms of the actual cost of media used for batch and fed-batch schedules the batch media is considerably less expensive. This result is expected for our process and arises from the fact that the nutrient media used for the fed-batch schedules is approximately 10 times more expensive than the media used for the batch schedules. The reason for the higher cost in this case is that the media used for the fed-batch process contains a number of costly additives not required in the batch process media. This is very obviously a significant difference in media cost. However, it is compensated for by the high average productivities achieved in the fed-batch schedules. This is reflected in the average production cost profiles for the batch and fed-batch schedules (Figure 4.20 & Figure 4.21).

4.1.1.6 Total Biological Waste Volume

Figure 4.24 and Figure 4.25 presents the benchmark data for the total waste (biologically active) volume generated over the duration of each batch and fed-batch production campaign. Since no biologically active waste is generated by either of the production vessels in batch or fed batch operating modes, the major waste contribution comes from the PGV. Or more precisely,

for purposes of benchmarking, it has been assumed that there can be no failure (contamination) of a production batch. Hence, there is no generation of biological waste from the production vessels. The issue of batch failures in the context of operating schedule robustness is dealt with later in Chapter V.

Considering just the batch schedules shown , there appears to be a degree of parity between the {1S3, 2P3}, {1S2, 2P4} and {1S3, 2P4} schedules, while the {1S2, 2P3} schedule produces considerably less waste. This in part can be attributed to the longer 10 batch production times for the 3 former schedules. Clearly, the longer a particular schedule runs for the more waste, over time, that will be generated. However, further reasons related to process kinetics and the particular schedule also exist.

As mentioned previously, when discussing media utilisation for our schedules, the longer the PGV or production vessel duration the greater will be the final cell density reached. In order to return the cell density to a pre-set minimum value for the next PGV cycle a proportionately larger volume of nutrient media will be required to achieve the required level of dilution. Also, the total volume of the PGV must not exceed the maximum volume specified for the PGV; a larger volume of the culture already in the PGV must be disposed to waste to accommodate the increased nutrient media volume. When, however, the PGV is to seed a production vessel, the waste volume is lessened because a significant volume of culture is now used as seed material for the next production vessel.

The volume of waste produced is also a function of the particular schedule chosen. For example, looking again at the {1S2, 2P3} schedule diagram (Figure 4.4) it is seen that a production vessel is always available to receive active culture from the PGV. Hence, the total volume of waste generated per PGV cycle is reduced, since a considerable volume of the PGV culture that would otherwise be disposed to waste is now used as seed material for the production vessel. A similar argument is applied to the {1S3, 2P3} schedule, the network diagram for which (Figure 4.6) shows the same balance between

the PGV and the production vessels. However, the same balance does not exits in the {1S2, 2P4} schedule. Figure 4.5 shows the network diagram for this schedule, from which it can be seen that every third PGV cycle results in the direct disposal of waste as neither production vessel is available to receive active culture from the PGV. The network diagram for the {1S3, 2P4} schedule, shown in Figure 4.7, also shows a balance between the PGV and production vessels. Although, in this case the predominant cause for the high waste volume is the longer PGV cycle duration resulting in the higher final cell densities.

The fed-batch schedule network diagram for {1S2, 2P7} schedule (Figure 4.8) shows that there are at least two PGV cycles for each production vessel cycle. Since no transfer of active culture can take place for these PGV cycles, the same cell density balancing argument must apply. Therefore, for the fed-batch schedules there will more instances where a greater volume of PGV culture has to be disposed of to waste. The network diagram for the {1S3, 2P7} schedule (Figure 4.9) shows that there is only the one PGV cycle for each production vessel cycle, where no transfer occurs. However, since the PGV duration in this schedule is 3 days, a higher final cell density can be expected. So for those cycles where no transfer occurs to a production vessel, the volume of waste generated will have to be proportionately higher than in the 2 day case.

4.2 Resource Allocation Profiles

The following results and discussion focus upon the process operator allocations over the duration of each of the schedules studied. For benchmarking purposes the initial size of the operator pool available for each of the configurations studied was fixed at 4 operators. The simulation model assumes that each of the operators is equally skilled and therefore selects operators from the pool randomly, so that each operator has an equal chance of selection for any given task.

4.2.1 Resource Allocation Procedure

The following provides an explanation of how process operator allocation is handled within the simulation model.

As stated previously process operators are selected randomly from the operator pool. When an operator is assigned to a particular activity, such as vessel filling, that operator resource is then fully assigned for the whole duration of the activity. Further, the same operator is 100% assigned to that task. So, for example, if a filling activity were to take 30 minutes then that operator would be assigned for 30 minutes and would be unavailable for any further activities during this period. Once the activity is completed the resource is returned to the operator pool for re-assignment. If an operator or operators are not available for any given activity requiring a resource then that activity will experience a processing delay until an operator resource becomes available.

The following illustrates the operator resource allocation sequence. The specific example used will be for the operation of a single fermenter/culture vessel.

4.2.1.1 Generic Operator Allocation Sequence

For any of the fermenter vessels used in this simulation model the sequence of operator allocation is shown below in Table 4.4. The first activity requiring a resource is the fill-with-culture. The operator/resource pool size is set at four and the number of assigned resource is one. The second activity is the fill-with-media. These two activities so far are separated in time and will not require a resource simultaneously. Consequently only a single resource is required for the second activity.

Sequence Order	Activity	Resource Required	Pool Size	No. Resources	Current pool Size
1	Fill-with-culture	True	4	1	3
2	Fill-with-media	True	4	1	3
3	Growth	False	4	0	4
4	Transfer	False	4	0	4
5	Fill-with-culture	False	4	0	4
6	Fill-with-media	True	4	1	3
7	Growth	False	4	0	4
8	Transfer	True	4	1	3

Table 4.4 Table showing a generic sequence of activities associated with the operation of a fermenter vessel and the corresponding level of resource allocation required for each activity.

The growth activity is the next in the activity sequence. Table 4.4 shows that no resource is assigned to this activity. The transfer activity in this example also does not require any resource to be assigned to it. This implies that no transfer of active culture is made to the next vessel in line. The sequence then repeats itself with the fifth, sixth and seventh activities. The final transfer activity in the above example does this time require a resource to be assigned to it. This implies that there is an actual transfer of active culture to the next vessel in line. The next vessel in line then goes through the same sequence of resource allocation.

It is important to note that the sequence shown in Table 4.4 is generic and will therefore be applicable to all the fermenter vessels in the simulation. Further, the allocation of resources for each active fermenter vessel will occur simultaneously limited only by the availability of resources.

4.2.2 Benchmarking Process Resource Allocation

Figure 4.26 shows the average peak process operator requirement over the duration of a production campaign employing the {1S2, 2P3} operating schedule. Each bar on this graph represents the peak or maximum number of process operators, taken from the existing operator pool, used during that day. It is clear that the maximum utilisation of the operator pool occurs over a four day period (16 to 20 days). The modal value for peak operator requirement appears to be 1. That is, the requirement for a single operator from the pool is the more common over the duration of this campaign, hence the operator pool is under utilised.

The frequency distribution shown in Figure 4.27 for the {1S2, 2P3} schedule provides a clearer picture of the operator pool utilisation. Ideally a distribution showing a positive skew (shifted to the right) would be sought after, implying greater utilisation of the existing operator pool. However, as is seen from Figure 4.27 a negative skew is apparent. Overall, this schedule makes the greatest use of 1 and 3 operators over the duration of the production campaign.

The maximum utilisation of the operator pool, over the 16 to 20 day period corresponds to that phase of the process when all the culture vessels and the PGV only are in use. It is important to remember that operator allocations occur, for the most part, between vessel growth and production cycles. So the operator allocation profiles represent the work being done to make each vessel ready for the next cycle. This includes preparing nutrient media feeds and transferring seeding cultures when needed. When a vessels is referred to as 'in use', the actual reference is to those vessels that require or will require some degree of resource allocation.

From the 20 day point there is a clear decline in the utilisation of the operator pool. Prior to the 20 day mark, the average peak operator allocation lies between 3 and 4 operators; from the 20 day mark onwards, there is a

downward shift in the average peak operator allocation to between 1 and 2 operators. This trend is expected and is attributed to the reduction in the number of culture vessels that are in use. Conversely, from the start of the campaign and as it continues, there is an increase in the utilisation of the operator pool due to increasing numbers of culture vessels coming into use. However, the pivotal point occurs during the 16 to 20 day period over which the PGV becomes active. From this point onwards, the number of culture vessels held as backups is reduced until only a single culture vessel is maintained in an active status behind the PGV. Then there is a brief period where only the PGV and a backup culture vessel are in use, following which the production vessels then sequentially come into use.

However, once both production vessels come into use, there are then effectively the same number of vessels in use as there were up to day 20 of the campaign. The data, however, points to a reduction in the average utilisation of the operator pool. This apparent reduction is explained by the fact that the production vessels, once in use, operate for a minimum of 3 days, while the culture vessels are operated for 2 days when in use. Further, there is an operating phase shift between the two production vessels, which is equivalent to the duration of the PGV. Hence, the production vessels will never be competing for the allocation process operators and the utilisation of the operator pool is low. There is, however, no phase shift for the culture vessels; they all operate in a concurrent manner and therefore create a greater demand upon the operator pool.

Similar peak allocation profiles for the {1S2,2P4}, {1S3,2P3} and {1S3,2P4} batch schedules are shown by Figure 4.28, 4.30 and 4.32 respectively. The associated frequency distributions are shown by Figure 4.29, 4.31 and 4.33 respectively.

Inspection of the remaining batch schedules reveals that each has an identical peak operator allocation pattern up to day 16. This observation is explained by the fact this initial 16 day period represents the culture stage of

each campaign. The operation of the culture stage is, in the case of the batch schedules, identical. In other words, the same number of culture vessel are used with the same vessel durations. Hence, the initial trend observed for each of the batch schedules will be identical.

A similar trend as that seen with the {1S2, 2P3} schedule is witnessed for each of the remaining schedules from day 20 onwards, that is to say, there is a decline in the peak number of operator allocated. The reasoning is again the same as that used for the {1S2, 2P3} schedule.

Although the peak allocation profiles for the remaining batch schedules appear very similar, a different picture emerges from the frequency distribution plots for each of these schedules.

In comparison to the {1S2, 2P3} schedule frequency distribution (Figure 4.27), the remaining batch schedules show a marked increase in the number of days during which there is a zero utilisation of the operator pool, i.e. when no operators are needed. This increase approximates to a doubling in the number of days where no operators are needed. During the culture stage (up to day 20),the peak allocation profiles are shown to be identical, therefore the total number of days that the operator pool remains idle must be the same in each schedule. It then follows that the differences between the schedules must arise as a result of the various combinations of PGV and production vessel durations, i.e. the individual production schedules.

The frequency distribution profiles also show a positive shift in the distribution of the data. This means that there is a shift towards a higher peak number of operators being allocated over the duration of each campaign.

The peak operator allocation profiles for both of the fed-batch configurations are shown by Figure 4.34 and Figure 4.36 for the {1S2, 2P7} and {1S3, 2P7} schedules respectively. The relevant frequency distribution profiles are shown by Figure 4.35 and Figure 4.37.

Unlike the peak operator allocation profiles described for the batch schedules previously, the fed-batch schedule profiles show a shorter initial period where the two profiles are identical. For the batch schedules, this parity was determined to be indicative of the culture stage of the overall production process. Similarly, for the fed-batch schedules the apparent initial agreement between the two profiles is related to the fact that this period represents the culture stage of the process. The shorter duration of this period is due to the particular operating protocol used for fed-batch operation. This refers to the fact that, for fed-batch operation, one of the culture vessels is used as a media storage vessel. Therefore, there is one less vessel to pass through before the PGV is reached and this is reflected in the shorter culture stage duration.

With either of the fed-batch schedules, as with the batch schedules previously, it is seen that the operator pool is seldom fully utilised, complete pool utilisation occurring on 3 and 2 days for the {1S2, 2P7} and {1S3, 2P7} schedules respectively. For the {1S2, 2P7} schedule, the frequency distribution profile (Figure 4.35) shows a smoother usage frequency for the operator pool than previously seen with the batch schedules. The {1S3, 2P7} schedule frequency distribution profile (Figure 4.37) shows a definite negative skew, indicating the under utilisation of the operator pool for the schedule used.

4.3 Summary

Experiments (simulations) have been carried out to determine the characteristic behaviour of 4 batch operating configuration schedules and 2 fed-batch operating configuration schedules. These schedules represent those schedules that are known to be achievable and for which real process data and knowledge have been made available.

Of the two process operating configurations presented above, the fed-batch system proved to be the most cost effective. This result is not unexpected for such a configuration which is currently the configuration of choice for the real process. This adds to the overall level of validity that can be attached to the simulation model used. Further model validity is afforded by the results attained for the average production costs. The values attained are of an acceptable order as suggested by process managers. In dealing with the issue of the validity of the model and the results developed from the model, it must be remembered that all of the outputs will lie within a 'window' of feasibility. In other words, there is no absolute answer for any of the performance parameters studied as no two simulation runs of the same schedule will give the same answer. It should be noted that the data presented represent averaged values over a number of simulation runs.

The initial characterisation of the configurations and schedules of interest provides a means of identifying operational areas that have further scope for possible streamlining. One such area is the reduction of vessel waiting time thereby maximising equipment utilisation. This would entail determining the causes or sites of process bottlenecks and the feasible solutions. The postulated solutions to such problems may then be tested by conducting 'what if' simulations and comparison with the benchmark process results. If the results from such 'what if' simulations or scenarios are found to result in improvements in a number of the relevant process performance measures then a strong case may exist for the implementation of the proposed measures on the real process.

In the following chapter a number of 'what if' scenarios are presented and evaluated. These relate to the reduction of vessel waiting times, the determination of the optimal size for the process operator pool and also how well the individual schedules cope with the occurrence of batch failures.

CHAPTER V

Variable Process Scenario Studies

5.0 Introduction

Following on from the characterisation of the batch and fed-batch process operating schedules of Chapter IV , the work presented in this chapter will examine the process issues that have been identified as amenable to further study. The relevant figures cited in this chapter are, for clarity, located in Appendix D.

The particular areas identified are the reduction of media transfer times from the media filtration unit, the enlargement of the media preparation capacity of the process, the determination of the optimal size of the available operator pool and finally the assessment of which of our current process schedules is the more tolerant to batch failures.

Each of the above stated areas of further study represent alternative process 'scenarios' which are simulated and compared against the process benchmark performance profiles.

5.1 Media Transfer Time Reduction: Results & Discussion

As determined from the initial process benchmarking studies of Chapter IV, there were two main factors that had the most significant effect upon the media transfer times and hence the vessel waiting times. These factors were the media filter unit filtration rate and the total media preparation capacity of the media preparation stage.

Simulation runs were carried out firstly to determine the effects, if any, of an increase in the media preparation capacity of the media preparation stage. This increase in capacity manifests itself as an increase in the maximum

capacity of MB1 from 3500 litres to 8000 litres. Accordingly, equipment cost adjustments are also made.

The next series of simulations then looked at the effects of an increased media filter unit filtration rate, the actual increase in the filter rate being approximately equivalent to twice the current rate. The third set of simulations then probed the effect of an increase in both the media preparation capacity and the media filter unit filtration rate. For convenience, each of these series of simulation runs are referred to as Scenario 1, 2 and 3 respectively. These simulations were conducted for each of the batch and fed-batch schedules referred to in Table 4.2.

Figure 5.1 compares the average wait time experienced by the PGV, operating under {1S2, 2P3} batch schedule, as a result of the different experimental scenarios. The benchmark profile reveals that the average amount of time that the PGV waits for media can be up to eight hours. This is particularly important when remembering that the media preparation process is not a sterile operation, so excessive delays can lead to the build up of endotoxic material. A number of the schedule profiles reveal a cyclic pattern in the PGV waiting times for the benchmark process. This pattern is related to the ability of the media preparation stage to meet the total media demand using the existing capacity. On those days where the waiting time profile is at a minimum, the total media volume requested can be prepared in either of the two media preparation vessels or via a combination of both vessels. Therefore, a reduction in the PGV waiting time is incurred.

It is apparent that significant reductions are attained with either an increase in the media preparation capacity or an increase in the filtration rate of the media filter unit. The greatest benefit is seen to be gained from a combination of these two measures, that is, increasing both the media preparation capacity and the filtration rate. An average waiting time of 3 hours is maintained as opposed to the eight hours of the original process.

Any decrease in waiting time achieved through an increase in filtration rate is self explanatory: the larger the volume of media that the filter unit can filter in unit time the quicker the media will reach its destination. With the increased media preparation capacity, there is no longer the need to wait for a media blend vessel of suitable capacity to become available. Media preparation can begin immediately. This reduces the PGV waiting time, since there is no longer a need to wait for the production vessels to receive media before media preparation for the PGV can begin. However, the media filter filtration rate does still pose a bottleneck. Similarly, the media preparation capacity poses a bottleneck in the increased filter unit filtration rate scenario: the capacity of one unit - in this case the filter - cannot be increased without increasing the other.

This is reflected in the third scenario (increased media preparation capacity and increased filter unit filtration rate) profile. The PGV waiting time achieved with this scenario is considerable lower than in any of the other scenarios. Similar trends are seen for the {1S2, 2P4}, {1S3, 2P3}, {1S3, 2P4}, {1S2, 2P7} and {1S3, 2P7} schedules (Figure 5.2 , 5.3, 5.4, 5.5, 5.6 respectively).

From the above interpretation of the results, the picture that emerges is that as far as the reduction of PGV media waiting times are concerned the favoured scenario is that of a combined increase in media preparation capacity and media filter unit filtration rate, i.e. Scenario 3. However, this is not the case when the media waiting times experienced by the production vessel (1D & 2D) for each scenario are examined. The average production vessel waiting times for each schedule and scenario are shown in Table 5.1.

Inspection of Table 5.1 shows that Scenario 2 is dominant in terms of reduced waiting times. In other words, an increase in the media filter unit filtration rate alone has a more significant impact than the combined increased media preparation capacity and increased filtration rate upon the production vessel waiting times. This is observed for each of the operating schedules studied. This result is attributed to the fact that the production

vessels have priority in media preparation and delivery. Therefore, media preparation capacity is not an issue. Ultimately, only the actual rate at which the prepared media is sterile filtered and delivered will have any bearing upon the waiting time experienced by each production vessel. In this case the higher the rate the shorter the waiting time.

	Production Vessel Average Waiting Time (hrs)							
	Benchmark		Scenario 1		Scenario 2		Scenario 3	
Schedule	1D	2D	1D	2D	1D	2D	1D	2D
1S2, 2P3	3.14	3.05	4.6	4.51	1.81	1.95	2.71	2.3
1S2, 2P4	3.36	3.12	4.02	4.01	1.99	1.87	2.42	2.48
1S3, 2P3	4.1	3.39	5.47	4.57	2.56	2.55	3.41	3.34
1S3, 2P4	4.25	4.14	6.31	5.82	2.49	2.52	3.5	3.47
1S2, 2P7	2.77	2.45	3.93	3.63	1.75	1.8	2.32	2.34
1S3, 2P7	3	3.61	4.52	4.52	2.02	1.88	2.79	2.75

Table 5.1 Summary table showing the average media waiting time experienced by either production vessel (1D & 2D) under the different process scenarios. Scenario 1 representing an increased media preparation capacity, Scenario 2 an increased media filter unit filtration rate and Scenario 3 a combined increase in media preparation capacity and increased filtration rate.

As indicated by Figure 5.1 and the profiles for the other schedules studied, Scenario 3 results in the better overall performance in terms of reduced PGV waiting times, while, Scenario 2 offers the better option for reduced production vessel waiting times. Clearly a choice must be made between these two scenarios. The final decision made will centre around the relative priorities that are attached to the PGV compared to the production vessels. Ultimately, any decision regarding process alterations will be gauged by their effects upon the production stage as whole and the resultant performance measures. Hence, based on this, Scenario 2 now becomes the favoured option.

The next course of action would be to compare the Scenario 2 against benchmark process performance measures such as the average product cost and the overall schedule productivity. Comparison profiles for the average

product cost and overall schedule productivity are shown in Figure 5.7 and Figure 5.8 respectively.

The average product cost comparison profile shows that a lower production cost is achieved with an increased media filter unit filtration rate only for the batch schedules: the fed-batch schedules show an increase over the benchmark values. This observation is explained as follows. The values for the average waiting time for the fed-batch schedules, shown in Table 5.1, represent only the average time taken for the initial transfer of nutrient media from the media preparation stage. All subsequent transfers of media originate from the cv1250 vessel which acts as an intermediate media storage vessel during fed-batch operation. The waiting times incurred in transferring media from the cv1250 to the production vessel is of the order of a few minutes on average. Therefore, a higher rated media filter unit will have a minimal effect upon the benchmark values. Consequently, a higher unit cost is incurred to account for the increase in fixed capital cost of a higher rate filter unit. This cost is not offset by any significant increase in schedule productivity.

The relative difference in the average production cost between the benchmark process and Scenario 2 process is slight in all cases. The limited performance enhancement attained via increasing the media filter unit filtration rate is explained as follows. Our performance measures are based upon the total number of units of product produced by each production vessel. Hence, any factor that increases the amount of time that the vessels are active will increase the amount of product produced. From Table 5.1 it can be seen that the saving in vessel wait time is between 1 and 2 hours when comparing the benchmark process to Scenario 2. For animal cell based processes, 1 or 2 hours extra production time has only a minimal effect due to the slow growth and production rates of animal cells in general. Also, recalling that the production vessels are allowed in all cases to complete their entire duration and are not stopped prematurely, then vessel waiting is not an issue as far as the production vessels are concerned. Hence we can account

for the close proximity of the benchmark and Scenario 2 processes in terms of the performance measures.

The effect of Scenario 2 upon the overall process performance parameters and specifically the production vessels does on the whole seem to be marginal. Given this information it would initially seem unlikely that the changes proposed by Scenario 2 would be implemented on the real process, since the simulation results show that the benefits from such a change are minimal. It may be argued that since there appears to be very little that can be done to greatly improve the production vessels performance, then efforts should be focused upon the improvement in the performance of the PGV. Any attempt at improving the PGV performance, as exemplified by Scenario 1, 2 and 3, will have a positive but still only marginal effect upon the overall process performance. The cautious standpoint is to ignore the results of Scenario 2 on the grounds that the observed small performance enhancement does not justify the major expense incurred with integrating a new media filter unit with a higher filtration rate into the existing process. It may then be argued that the financial resources that may have been allocated to achieving the integration of the new unit could be directed into other research and development oriented areas, such as media development or cell line enhancement rather than operational areas. The financial resources involved in such an effort are then targeted more efficiently.

However, although the benefits in terms of cost reduction and increased plant productivity appear to be minimal, the benefits in terms of greater process operability must also be considered. Improved process operability will generally result in increased efficiencies in terms of asset utilisation, that is to say production vessels spend less time idle between batches. Further, the daily schedules are also maintained comfortably within the pre-defined operating time frame. Process operator usage during a given day will not change; however, the reduction of waiting times has the effect of reducing instances where over time work may be required.

5.2 Determination of Optimum Operator Pool Size: Results & Discussion

The emphasis of this specific section will be to present a means by which the optimal operator pool size may be judged for each of the schedules studied. This was enabled by conducting a number of simulation runs for each schedule with a different operator pool size for each simulation. The range of the pool sizes considered was from 0 to 5 operators, although data for the zero pool size is not available since any such simulation would be in-operable.

Initially, considering the operator allocation profiles introduced in Chapter IV, it is apparent that the demand for process operators from the operator pool is transient: for any particular schedule, as time and the process advances, the peak daily requirement for process operators changes. This in effect represents the changing work content of the process and the specific schedule employed. The inference from this observation is that the optimal scheduling of the available operator resource is best achieved via a proactive approach to meeting the operator resource demands of the process.

Figures 5.10 , 5.11, 5.12 show the operator allocation profile for the {1S2, 2P3} schedule with an operator pool of 2, 3, and 5 available operators respectively, while the operator allocation profile for the same schedule with 4 operators in the pool is shown by Figure 4.26. Figure 5.13 shows the frequency distribution profiles for the peak daily operator allocation for the different pool sizes studied for the same schedule. Each peak represents the total number of times, on a daily basis, that that number of operators from a given pool size are used. So, for example, for the pool size of 2 operators, the pool is completely utilised for approximately 48% of the campaign time, while the pool is 50% (1 operator) utilised for approximately 35% of the campaign time.

From Figure 5.13 it is clear that an operator pool size of 5 is no advantage over that of a pool consisting of 4 operators since both the frequency distribution and operator allocation profiles are identical. Also, Figure 5.13 shows that the maximum possible daily demand for operator resource is 4. This is indicated by the fact that when the pool size is 5 operators there are no instances when the peak daily demand reaches 5 operators. So, as far as the {1S2, 2P3} schedule is concerned, the maximum daily work content is met by 4 operators.

The most significant change in the profile of the frequency distribution, shown in Figure 5.13, occurs with the increase in operator pool size from 2 to 3 operators. An approximate halving in the frequency of 2 operators being the daily peak number of operators is seen. This is mirrored by an equivalent increase in the peak daily requirement frequency of 3 operators. The implication in this case being that the daily work content or work load of the current process is transient between that met by 2 and 3 operators. In comparison, however, an increase in the available operator pool size from 3 to 4 operators shows a less marked change in the frequency distribution profile. The decrease in the peak daily requirement for 3 operators is compensated for by an equivalent increase in the requirement for 4 operators. However, examination of the operator allocation profile for an operator pool size of 4 shows (Figure 4.26) that maximum utilisation of the pool occurs only on 3 occasions for the whole of the duration of the process.

An interim conclusion from the frequency distribution profiles for the {1S2, 2P3} schedule suggests that an operator pool size of 3 operators best meets the daily process demand for operator resources. This observation is given further credibility when delays in the availability of operators to assist in the transfer of media to the PGV and production vessels are considered.

Table 5.2 shows the average delay, in hours, resulting from a limitation in the availability of operators to assist in filling culture and production vessels with nutrient media. From Table 5.2 it is clear that the minimum number of

operators required in the operator pool is 2 in order to minimise any resource related delay in filling the vessels with nutrient media. No limitation is seen with either of the production vessels when the operator pool consists of only a single operator. This is explained by the fact that both production vessels have priority for media preparation. So once media has been prepared using the single operator, the same operator is then available to transfer the media resulting in no delay. Also, for this schedule, the production vessels are not competing for operator resource with any other vessel.

Pool Size	cv100	cv600	cv1250	PGV	1D	2D
1	0.04 (+0.30, -0.04)	0.25 (+1.18, -0.25)	1.47 (+1.29, -1.52)	0.05 (+0.48, -0.05)	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0

Table 5.2 Summary table showing the average time delay, in hours, suffered by the culture and production vessels as a result of a limitation in the size of the available operator pool for the {1S2, 2P3} schedule. Since the non-zero values are averages the range of the data is also given.

For the {1S2, 2P3} schedule it seems that a lower threshold level, at least, is definable for the number of operators to have in the operator pool. Table 5.2 suggests that this level should be 2 operators. The operator allocation and frequency distribution profiles for this schedule (Figures 5.10 and 5.13 respectively) suggest that, for 2 operators in the pool, the operator pool is fully utilised on a daily basis for 49% of the duration of the process. This value may not seem particularly impressive when judged against resource utilisation for other manufacturing processes. However, it must be remembered that the temporal dynamics of this process is such that long periods of resource (operator) inactivity are unavoidably incurred. If the profiles for the operator pool size of 3 are considered then it is seen that there is a significant decrease in the allocation of 2 operators in favour of 3

operators. This implies that the work content on those days were a peak allocation of 2 operators is replaced by 3 operators is better managed with 3 operators. It then follows that the operator pool of size 2 is over-loaded on those days were 3 operators would be needed. In comparison, when the pool size is increased from 3 to 4 operators there are only 3 days on which a peak allocation of 3 operators is replaced by 4 operators. Although, this represents an over-load for 3 operators in the pool its occurrence is not of the same size as that seen with 2 operators.

Therefore, although an operator pool size of 2 operators offers zero delay in making available the necessary operator resources for media transfer, a pool size of 3 operators is operationally more acceptable.

The peak operator allocation profiles for the {1S2, 2P4} operating schedule are shown in Figure 5.14, 5.15, 5.16 for operator pool sizes of 2, 3 and 5 respectively. The daily peak operator allocation frequency distribution profile for the entire range of operator pool sizes is shown in Figure 5.17.

As with the analysis of the previous schedule, the allocation and frequency distribution profiles for the {1S2, 2P4} schedule may be interpreted similarly. Again, the peak daily work load of this schedule does not exceed that which can be carried out by 4 operators, so a fifth operator is not needed. As in the {1S2, 2P3} schedule, the frequency of days were an operator pool of size 4 is fully utilised during any part of the day is small in comparison to the same frequency for pool sizes of 3 and 2 operators. Overlaying the operator allocation profile for the pool size of 2 with that of the pool size of 3 (Figure 5.14 and Figure 5.15) shows a 40% decrease in the frequency of daily peak allocation of 2 operators. This decrease is accounted for directly by an increase in the frequency of daily peak allocation of 3 operators. This implies that on those days were 3 operators replace 2 operators as the peak number of operators used there is a better distribution of the work load.

The lower threshold size of the operator pool is determined in a manner similar to that for the previous schedule, in terms of the number of operators required to reduce or eliminate any resource availability related delay in the transfer of nutrient media to the culture and production vessels. As with the previous schedule results (Table 5.2) the limiting size for the {1S2, 2P4} schedule was again 2 operators. Anything below results in the incursion of operator availability related time delays. The results for this are shown in Table 5.3 where only the data for a pool size of 1 operator is shown.

Pool Size	cv100	cv600	cv1250	PGV	1D	2D
1	0.04 (+0.30, -0.04)	0.20 (+0.81, -0.20)	1.17 (+1.99, -1.17)	0.13 (+0.40, -0.13)	0.00	0.00

Table 5.3 Summary table showing the average time delay, in hours, suffered by the culture and production vessels when only a single operator is available for the {1S2, 2P4} schedule. The values for pool sizes 2, 3, 4 and 5 have not been shown as they are zero in all cases. The range of the data are also shown where relevant.

It would seem, for the {1S2, 2P4} schedule, that an operator pool size ranging between 2 to 4 operators is best suited to handling the work load of this schedule and minimising transfer delays due to limitations in available operators.

The remaining two batch and the two fed-batch operating schedules are considered similarly. Figure 5.18 to 5.20 present the peak operator allocation profiles for the {1S3, 2P3} schedule with an operator pool size of 2, 3 and 5 operators respectively. The corresponding allocation profiles for the {1S3, 2P4} schedule are shown by Figure 5.22 to 5.24. Examination of the daily peak operator allocation frequency distributions for the batch schedules {1S3, 2P3} and {1S3, 2P4} (Figure 5.21 and Figure 5.25 respectively) again yields a similar picture to that of the previous two batch schedules studied. It emerges from the allocation and frequency distribution profiles for these batch schedules that an operator pool size of 3 provides the better overall capacity to handle the work load as both schedules evolve. As with the previous schedules, the effect of the operator pool size upon the delay

experienced in transferring nutrient media is again evaluated. The results of this and the results for the fed-batch operating schedules are presented in Table 5.4 . Again, for the same reason as in the preceding schedule, only the data for an operator pool size of 1 are shown for each schedule and operating configuration.

Schedule	cv100	cv600	cv1250	PGV	1D	2D
{1S3,2P3}	0.04 (+0.30, -0.04)	0.11 (+0.23, -0.11)	0.80 (+2.79, -0.80)	0.22 (+1.29, -0.22)	0.00	0.00
{1S3,2P4}	0.12 (+0.82, -0.12)	0.12 (+0.21, -0.12)	0.80 (+2.80, -0.80)	0.04 (+0.49, -0.04)	0.00	0.00
{1S2,2P7}	0.00	0.52 (+2.07, -0.52)	_	0.26 (+1.44, -0.26)	0.01 (+0.18, -0.01)	0.16 (+2.44, -0.16)
{1S3,2P7}	0.10 (+0.40, -0.10)	0.32 (+1.88, -0.32)	_	0.03 (+0.17, -0.03)	0.16 (+3.04, -0.16)	0.18 (+3.01, -0.18)

Table 5.4 Summary table showing the average time delay, in hours, suffered by the culture and production vessels when only a single operator is available. The values for pool sizes 2, 3, 4 and 5 have not been shown as they are zero in all cases. Where relevant the range of the data has been presented. No values exist for the cv1250 vessel in either of the fed-batch schedules since this vessel is used as a media storage vessel.

The operator allocation profiles for the fed-batch {1S2, 2P7} and {1S3, 2P7} schedules are illustrated in Figure 5.26 to 5.28 and Figure 5.30 to 5.32 respectively. The respective frequency distribution profiles are shown in Figure 5.29 and Figure 5.33.

With both fed-batch operator allocation profiles, it is apparent, at least for those profiles for pool sizes 3 and above, that the production stage of the process has a higher degree of work content than was seen with the batch schedule profiles. This positive skew in the work load distribution for fed-batch is to be expected since each batch of final product is generated through 3 fed-batch cycles. Each cycle requires operator resource in order to be carried out.

As with the previous batch schedules studied, there seems to be little point in maintaining an operator pool size above 3 operators and definitely no need to

have 5 operators. Although there is a daily peak concurrent work load demand equating to 4 operators for both of the fed-batch schedules, these occur early on and at a low frequency. The results from the study of the nutrient media transfer delay times for each pool size reveals the same trend for both of the fed-batch schedules as has already been seen with the batch schedules. The results for the average incurred delay due to unavailability of operators is shown in Table 5.4 above. Again, it is seen that 2 or more operators offer no delay in nutrient transfer times.

Although each of the schedules is different in performance terms, there appears to be a great deal of parity in the most suitable number of operator resources available in the operator pool. The general consensus is that the minimum level should be 2 operators in all cases. While the maximum number should be flexible between 3 and 4 operators depending upon expected demand and other operational factors including general process safety.

5.3 Operating Schedule Sensitivity to Batch Failure: Results & Discussion

Simulation runs were conducted for each of the 4 batch operating configuration schedules and the 2 fed-batch operating configuration schedules to determine the response of each of these schedules to the introduction of batch failures. A batch failure in this case is a contaminated production batch which requires disposal to waste and the cleaning and sterilisation of the production vessels before a new batch can be started.

As the intention in this section is to describe how the various schedules recover from an occurrence of a batch failure, such failures are introduced manually at the same points, equivalent to the second batches from both production vessels, for each schedule considered. All failures were assumed to occur or be detected effectively at the start of each batch.

Two separate schedule recovery polices were evaluated. These recovery policies are represented by rules (heuristics) that govern how active culture material from the PGV will be re-scheduled to a production vessel that has just suffered a batch failure, once this vessel has been cleaned and sterilised.

The first of these heuristics defines the need to maintain the overall sequencing of the production vessels with the selected schedule. This means that the normal scheduling of culture from the PGV, where alternate transfers are made first to the 1D production vessel and then to the 2D vessel and back to the 1D vessel again, is maintained. This process rule is referred to as Heuristic 1.

The second heuristic, Heuristic 2, defines the immediate re-scheduling of the next available batch of active culture from the PGV to the production vessel requiring it, irrespective of the original destination for this batch of seed culture from the PGV.

Figure 5.34 compares the total cumulative amount of product made by batch production campaigns working with a {1S2, 2P3} schedule with and without batch failures, using the two different schedule recovery heuristics. The time axis for Figure 5.34 shows the cumulative time between successive batches. This basically represents the time taken from the start of one successful batch in one production vessel to the start of the next successful batch in the same vessel. It will hence be composed of the duration of the production batch, the cleaning time associated with preparing the vessel for the next batch and the characteristic idle time associated with the particular schedule in use. When batch failures occur, the cumulative time will also include the additional cleaning time incurred and any additional idle time. For each of the profiles a production stage start time is given as a basis for reference. This basis represents the time at which the first batch is started in each case.

As would be expected, a shift along the time axis is witnessed for Heuristic 1 and 2. This shift is seen with all the remaining profiles for the schedules

studied (see Figure 5.35, 5.36 and 5.37 for the batch schedules and Figure 5.38 and 5.39 for the fed-batch schedules). This displacement results from the incursion of additional vessel cleaning and vessel idle time. The severity of the additional delay is dependant upon both the schedule and recovery policy in use. The general trend seen with these profiles is that Heuristic 1 produces the greater delay.

Heuristic 1 states that when a batch of product in any production vessel is contaminated then the next scheduled batch of active seed culture from the PGV be made available to the affected production vessel. The idle time experienced as a result of the batch failure, after the vessel has been cleaned and sterilised is in the range of 3 to 5 days depending upon the schedule. While for Heuristic 2, which states that when a batch of product in any production vessel is contaminated then the next immediately available batch of active seed culture from the PGV be made available to the affected production vessel, the idle time experienced due to batch failure ranges from 1 to 2 days. Table 5.5 summarises the idle times experienced by a production vessel, after the vessel has been cleaned and sterilised, that has experienced a batch failure.

Heuristic 2 provides a faster vessel turn round time than Heuristic 1 because, assuming that the contaminated production vessel has been cleaned and sterilised, then it is only waiting for the transfer of inocula from the PGV.

Schedule	Benchmark Idle Time (days)	Heuristic 1 Idle Time (days)	Heuristic 2 Idle Time (1days)
1S2, 2P3	0	3	1
1S2, 2P4	1	3	1
1S3, 2P3	2	5	2
1S3, 2P4	1	5	2
1S2, 2P7	0	3	1
1S3, 2P7	1	5	2

Table 5.5 Table summarising the idle times experienced by a production vessel that has suffered a batch failure. Different idle times values are obtained depending upon the type of schedule recovery heuristics applied each time a failure occurs for each operating schedule. The idle time values for the benchmark process are presented for comparison.

The values given in Table 5.5 do not represent the idle times that will experienced between each successive batch during normal operation. They apply only to that batch which is contaminated. When a batch failure occurs for one production vessel there is a 'knock on' effect upon idle time of the other production vessel. This is evident only when using Heuristic 2 as the recovery policy. When a contamination arises, the next available batch of active seed culture from the PGV is made available to the contaminated vessel. However, this batch of seed culture would normally have been destined for the other production vessel. Therefore the normal idle time experienced by currently uncontaminated and available vessel is further compounded and hence the 'knock on' effect that is observed. A further explanation is given by considering the resultant network diagrams for the {1S2, 2P3} schedule when employing Heuristic 1 and Heuristic 2, shown in Figure 5.40.

The network diagram where Heuristic 1 has been employed shows that for a batch failure in either of the production vessels, there is no increase in the idle time suffered by the next production vessel above that which it would experience under normal operating conditions (no batch failures). This is due to the fact that with Heuristic 1, the transfer schedule of PGV batches is maintained. Therefore the next production vessel in line will receive the

scheduled batch of inoculum from the PGV with no additional idle time incurred, at the expense of the contaminated vessel.

However, the network diagram where Heuristic 2 has been used shows that although there is a reduction in the idle time experienced by the vessel with the failed batch, the other production vessel experiences an increased idle time. This idle time is greater than the idle time it would experience under normal operating conditions. This is also shown in Table 5.5, where for the {1S2, 2P3} schedule the idle time has increased from zero to 1 day when using Heuristic 2.

The {1S2, 2P3}, {1S3, 2P3}, {1S3, 2P4} schedules all show this behaviour in a cyclic pattern: when the product batch in the 1D production vessel fails, the 2D vessel experiences an increased idle time. When the product batch in the 2D vessel fails the 1D vessel experiences the same increase in idle time as was previously experienced by the 2D vessel. This is seen to occur with both of the recovery policies employed.

The {1S2, 2P4} schedule, however, does not show this cyclic pattern for both recovery heuristics. Instead, the cyclic pattern is seen only when Heuristic 1 is employed. The idle time that results from a batch failure in the 1D vessel is shown as 1 day in Table 5.5 while the 'knock on' effect upon the 2D vessel is 3 days. If the cyclic pattern described earlier was to hold then the expectation is that when a failure occurs in the 2D vessel the 'knock on' effect will be 3 days idle time for the 1D vessel and 1 day for the 2D. However, Figure 5.41 shows that for Heuristic 2, after the second contamination, both vessels experience only a 1 day idle time. Effectively, the schedule displays a pattern of idle time equivalent to that seen with the normal operating schedule.

Similarly, both fed-batch schedules do not exhibit the same cyclic pattern of collateral idle time incursion for both recovery policies. Also, as with the {1S2, 2P4} schedule, the idle time values for the fed-batch schedules revert to those of the normal schedule.

The observed difference between the 3 batch schedules with a cyclic pattern of collateral idle time incursion and the 1 batch schedule and the 2 fed-batch schedules without this behaviour can in part be accounted for by considering the synchronous nature of these schedules. An explanation of a synchronous (balanced) schedule was presented earlier in Chapter II and Chapter IV. However, to re-cap, a synchronised schedule is one where the seeding pattern from the PGV exactly matches the demand for active culture (inocula) from the production vessel and hence results in no idle time.

Of the 3 schedules where this cyclic behaviour occurs, the {1S2, 2P3} schedule is synchronous, while the {1S3, 2P4} and {1S3, 2P3} schedules are partially balanced. On no occasion is any PGV batch disposed of to waste, although there is an incursion of a production vessel idle time. In the remaining 3 schedules, a number of active culture batches from the PGV are disposed to waste because neither of the production vessels are available to receive them, during normal operation. However, when batch failures occur, these 'surplus' batches of inocula from the PGV form a buffer. Consequently in the event that a batch failure does occur, specifically in the second production vessel, then the otherwise wasted batch of inocula from the PGV is used to re-seed the production vessel. This reduces significantly the idle time that would have otherwise been experienced by both production vessels.

It would appear from the results obtained thus far, that as far as schedule robustness to batch failures is concerned, operating asynchronous (unbalanced) schedules with recovery Heuristic 2 offers the lower incurred idle time penalties. This has the benefit of reducing the detrimental effect upon the overall schedule productivity that is necessarily incurred from additional vessel idle times.

Closer examination of the production vessel sequencing reveals that with Heuristic 1 the sequencing of the production is not maintained, although the schedule for the transfers of active seed culture from the PGV is maintained.

In the case of Heuristic 2 the production vessel sequence is maintained, i.e. 1D, 2D, 1D, 2D and so on, but the seed culture transfer schedule is not. The importance of the vessel sequencing arises from the need to maintain the operation of the process in accordance with documentation describing the detailed operation of each production campaign. When a new production campaign is planned a lengthy process of documentation is required for regulatory purposes, to define exactly how the process will be operated, which vessels will be used, their sequencing and the flow of batches between them.

5.4 Summary

Reduction in the media waiting times experienced by the PGV and production vessels have been shown to be achievable via three different process scenarios. The first, Scenario 1, is to increase the media preparation capacity of the process. The second, Scenario 2, is to increase the filtration rate of media filter unit and the third, Scenario 3, to combine the increase in media preparation capacity with the increased filtration rate of the media filter unit. Benefits are realised, mainly from Scenario 2 and 3, in terms of the overall schedule productivity and average production costs. However, these benefits represent only marginal increases over the benchmark process schedules. Hence, the sensitivity of the benchmark schedules performance to high vessel waiting times can be assumed to be low.

The underlying inference then is that although relatively large waiting times are evident in the benchmark process schedules and that technical solutions exist to reduce these waiting times, the cost of such ventures may not necessarily be justified by the returns in terms of overall process performance.

Simulations have been carried out to determine the optimum operator staffing levels for each of the process schedules defined previously. In all cases the analysis has been based upon evaluating the daily peak concurrent work

loads and how these equate to the available number of process operators. Further, the delay in transferring nutrient media to the culture and production vessels as a result of a limitation in the available operators has been evaluated. The results show that for all the schedules studied a minimum number of two operators is required to avoid delays in nutrient media transfer times. The maximum number of operators needed for each schedule is determined to be between 3 and 4 operators depending upon the particular stage of the process. This is because the work load of each schedule is shown to vary according to the particular process stage and therefore, the operator requirement will vary accordingly.

As to whether 2, 3 or 4 operators are maintained or not would ultimately become determined by operational factors, such as, safety and other areas of process operation that have not explicitly been accounted for in estimating the process work load. Irrespective of the schedule under consideration, such operational factors have a major bearing upon any final decision as to the best (optimal) operator pool size. All the allocation profiles thus far presented show no constant level throughout, although localised predictions are possible. These present process managers with sufficient information with which to make judgements about the best (optimal) way in which to deploy the available resources on any given day of operation and maintain the highest degree of flexibility possible.

It has been shown that in determining the optimal production pattern to batch failures the method of vessel recovery used (Heuristic 1 or 2) has a significant impact upon the overall schedule productivity, the overall schedule productivity being measured indirectly through the total amount of product made over the cumulative production duration.

The difference in the two recovery policies used is seen in terms of increased production vessel idle times over the normally expected values. These idle times all have a detrimental effect upon the cumulative productivity of any schedule. Further, the two policies have an effect upon the sequencing of the

production vessels which can present regulatory difficulties as the overall operation of the process may now not be following documented protocols for that campaign.

The analytical process model that is the subject of Chapter II has also been used to explore the sensitivity of optimal production patterns to batch failures. In this case only the schedule recovery policy equivalent to Heuristic 2 was considered. The simulation results show a high degree of convergence with those of the analytical model as far as this recovery policy is concerned. Also, for comparison, a second recovery policy was evaluated (Heuristic 1). The simulation model results for Heuristic 1 further strengthened legitimacy of Heuristic 2 as the better recovery policy, particularly for schedules that generate surplus PGV batches as predicted by the analytical model.

CHAPTER VI

Conclusions and Recommendations for Future Work

6.0 Concluding Discussion

In the first instance, this thesis presents a formulation of an analytical model describing a fixed existing monoclonal antibody manufacturing facility. This model is used to provide an initial means by which competing process operating schedules may be screened for optimal economic return and process operability, based upon the concept of balanced fermenter utilisation. Further, the model is applied to test a commonly accepted heuristic that the production phase should be selected to be as long as possible. These issues are evaluated using linear and non-linear antibody accumulation models. The model is further extended to consider the robustness of optimal process patterns to batch failures.

From the analytical model it is concluded that operation of the production phase for as long as possible does not necessarily yield optimum economic return for either the linear or non-linear antibody accumulation models. In the linear case, optimum economic return was found to be associated with a balanced fermenter train, while the non-linear case suggested that an unbalanced fermenter train might, for certain fermenter vessel configurations, offer some marginal financial gain.

A dynamic simulation model has been developed to represent the material and resource flows between the process stages and the unit operations in those stages that result from specific fermenter vessel operating schedules for the antibody production process that is the subject of the analytical model. The dynamic and graphical nature of the simulation model allows process bottlenecks to be highlighted and resource allocations to be probed and

charted in real time. Once bottlenecks have been identified, possible solutions can be evaluated against benchmark performance data.

Unlike the majority of process simulators currently available, this simulation model is not based upon complex unit operation and physiological process models. Rather, each unit operation is defined by a set of connected process activities. These activities define the operational logic of the unit operations concerned. By differentiating unit operations into discrete processing activities, accurate assignment of process resources (operators and utilities) and costs are enabled relatively easily.

The current process activities that have been defined form the initial basis for a library of common process activities. With a sufficiently large library many different unit operations can be defined simply by connecting the relevant activities together in a logical format.

The benefit of such an approach is to provide end users with a greater understanding of how the simulation model works and therefore enable these users to adapt the model as dictated by real process changes with minimal effort and programming knowledge.

The simulation model as it stands represents the basics of an interactive strategic and training tool for process operators and managers. The strategic element arises out of the flexibility of the simulation in rapidly evaluating different process scenarios in terms of performance parameters and also to gauge operational convenience. Also, the potential exists for providing process operators with a virtual training ground.

Using the simulation model, 6 different operating schedules have currently been evaluated to determine benchmarking performance statistics (see Chapter IV).

Comparison of the simulation results with those presented in the analytical model show a good agreement. Although certain differences between the two do exist it must be remembered that the analytical model is a simplistic representation of what is a complex manufacturing process. A specific difference that exists is the result for the {1S2, 2P4} schedule. The simulation results show it to be more cost effective than the {1S2, 2P3} schedule while the linear model predicts the reverse of this. This is attributed to the relative simplicity of the product accumulation sub-model used in the analytical model.

Further, the analytical model assumes that for dealing with failed (contaminated) production batches, the first available PGV batch be used to re-inoculate the production vessel, assuming the vessel has been cleaned and sterilised previously. This recovery procedure is only an option for those production patterns with redundant PGV cycles. This assumption is verified by simulation results which show that this recovery procedure (known as Heuristic 2 in the simulation trials) in conjunction with the {1S2, 2P4}, {1S2, 2P7} and {1S3, 2P7} production schedules, all of which have redundant PGV cycles, results in a rapid restoration of the original operating schedule profile. For comparison, a second feasible recovery policy is also evaluated whereby the contaminated production vessel is forced to wait for the PGV batch that is scheduled for it rather than receiving the next available PGV batch.

Overall, the analytical model formulation holds up well to comparison with the simulation data. The analytical model provides a useful tool for the initial short-listing of possible operating schedules on the grounds of optimal financial return. In comparison to the often complex and mathematically involved analytical models presented in the literature, this model presents a simple, tractable and industrially applicable optimisation paradigm.

As a result of the simulation studies that have been carried out on the feasible operating schedules presented in this thesis, a final recommendation

is presented detailing which operating schedule offers the best overall performance.

The simulation results have verified for this process the paradigm of fed-batch operation over batch operation as a significantly more productive operating strategy. This is illustrated most vividly by Figure 4.1 which shows the total cumulative amount of product made over the duration of each schedule. Both fed-batch schedules are far more productive than any of the batch schedules studied. If speed to market were a major concern then the {1S2, 2P7} schedule presents the most expedient route with a 25 day makespan for approximately $1x10^7$ units of product compared to 32 days with the {1S3, 2P7} schedule (see Table 4.3 or Figure 4.1).

Two fed-batch schedules have been studied. These are the {1S2, 2P7} and {1S3, 2P7} schedules. Of these two schedules the {1S2, 2P7} pattern offers the better overall schedule productivity and the lower unit cost of production, as shown by Figure 4.13 and 4.21 respectively. Although the average vessel productivity for both of these schedules is almost identical, the fact that the {1S2, 2P7} schedule has a higher overall schedule productivity indicates that this schedule is more efficient at production vessel utilisation. This is also indicated by the fact that the {1S2, 2P7} schedule has no production vessel idle time.

Both fed-batch schedules also fall into the group of schedules capable of rapid recovery of operating schedule profile after occurrence of a batch failure or failures. Therefore, the {1S2, 2P7} schedule is also a robust schedule as far as batch failures are concerned.

The debottling studies related to decreasing production vessel media waiting times show that both fed batch schedules have shorter waiting times than any of the other schedules. Further, the {1S2, 2P7} schedule, again has the lowest waiting times of all.

Analysis of the operator pool size reveals that the minimum number of operators in the operator pool for all schedules should be 2. For all the schedules considered, a pool size of 2 operators represents the maximum number required to eliminate media transfer delays due to unavailability of operator resources. However, for health and safety reasons 2 operators would not be feasible. The general conclusion that arises from an operator allocation analysis is that the minimum level should be 2 in all cases; however, bearing in mind safety factors, the actual number of operators available should vary between 3 and 4.

To summarise, the fed-batch {1S2, 2P7} operating schedule, employing Heuristic 2 as a failed batch recovery policy with a staffing level of 3 operators on a permanent basis and a fourth available when required, currently offers the best (optimal) process operating schedule.

6.1 Recommendations for Future Work

1. The simulation of operator resource availability was based upon a random assignment of operators to activities during any given day. This assignment did not take into account factors such as statutory breaks. Although the facility existed to assign operators with fixed working times and break periods (known as temporal scheduling), this facility was not used due to a flaw in its proprietary design.

2. The **cleaning** activity detail needs to be extended to include the various stages associated with vessel cleaning and sterilisation. The primary reason for not extending the design of the **cleaning** activity is related to the flaw in the design of the temporal scheduler stated above.

3. The messaging structure of the current simulation model is passive. Despite messages being generated that require the user to initiate a given action, e.g. begin media preparation for a given vessel or vessels, the user can ignore these intentionally or unintentionally. The messaging system

needs to be more active to either prevent this or inform the user of possible consequences.

4. The current simulation as described in this thesis deals only with the primary processing of product with no inclusion of downstream product recovery processes. The current simulation model does define a number of the unit operations involved in downstream processing; however, these have not been fully developed and implemented into a running simulation. Product recovery can account for up to 50% of the final product cost. Therefore, the recovery stage represents an equally important stage of the overall manufacturing process that must be made amenable to study and optimisation where necessary.

5. Current trends in the chemicals and biopharmaceuticals manufacturing industries having been moving away from single product purpose built manufacturing facilities. The focus has fallen increasingly upon product flexibility of existing facilities. The current simulation model can be modified to allow the operational evaluation of the current process resource inventory for multi-product (flowshop) processing. Simulating multi-product operation within an existing single product facility can aid in drafting standard operating procedures and assessing possible resource and segregation conflicts that may arise. Also, importantly such a simulation model can also be used to convince regulators to licence the facility for multi-product operation.

6. The work presented in this thesis deals exclusively with production schedules for two competing operating strategies, namely batch and fed-batch. However, at least two other operating strategies exit that are variants of batch and fed-batch operation. These are batch draw and fill and fed-batch draw and fill operation. Further work in evaluating these strategies can be done.

7. All the schedules evaluated using this simulation model have taken the vessel cleaning and sterilisation time to be 1 day. Further work investigating the affect of increasing this time to 2 or more days upon the performance measures already evaluated is needed.

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APPENDIX A (Chapter II Figures)

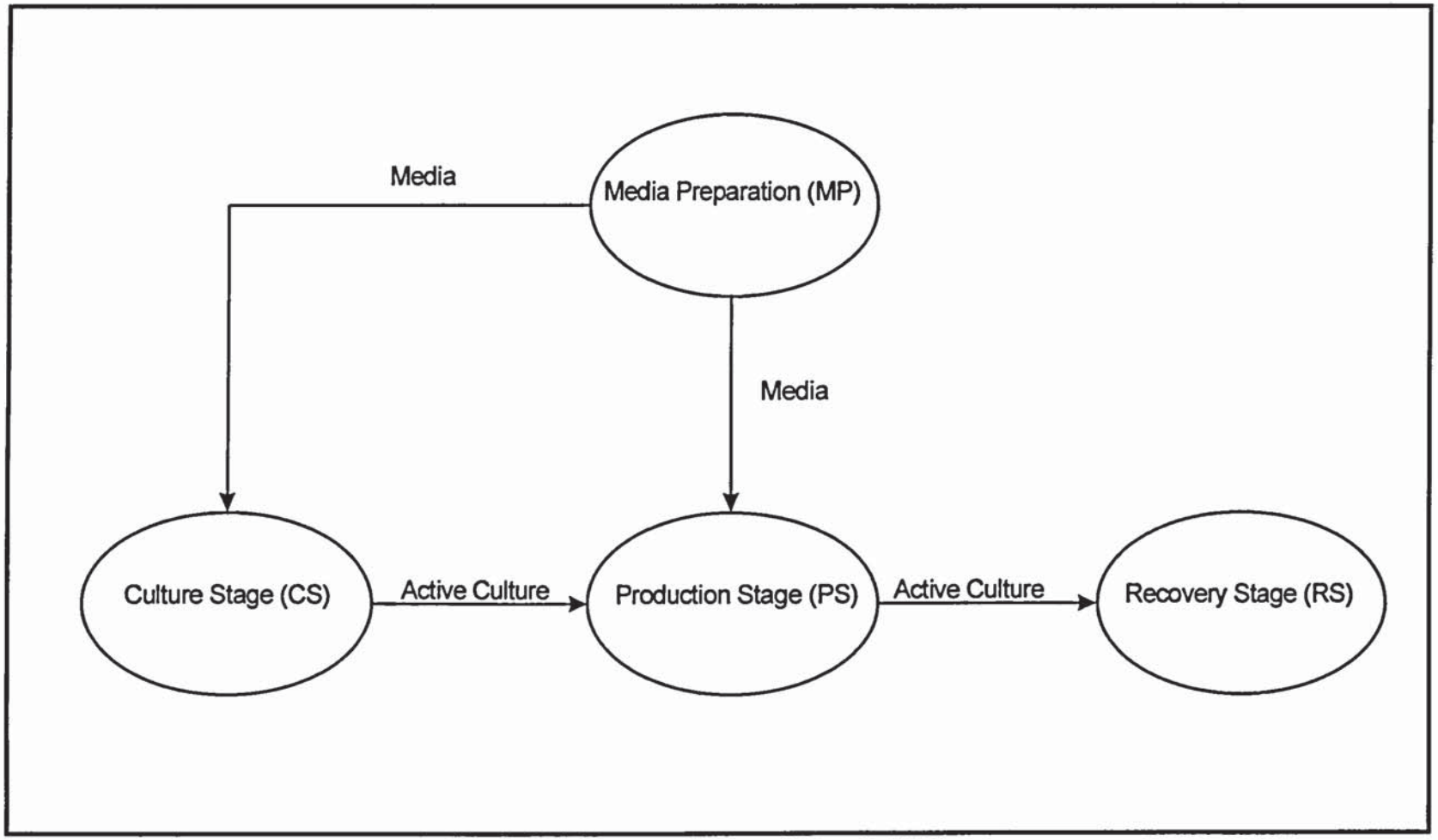

Figure 2.1 A generalised schematic diagram showing the main process stages of an existing monoclonal antibody manufacturing facility and the material flow relationship between those stages.

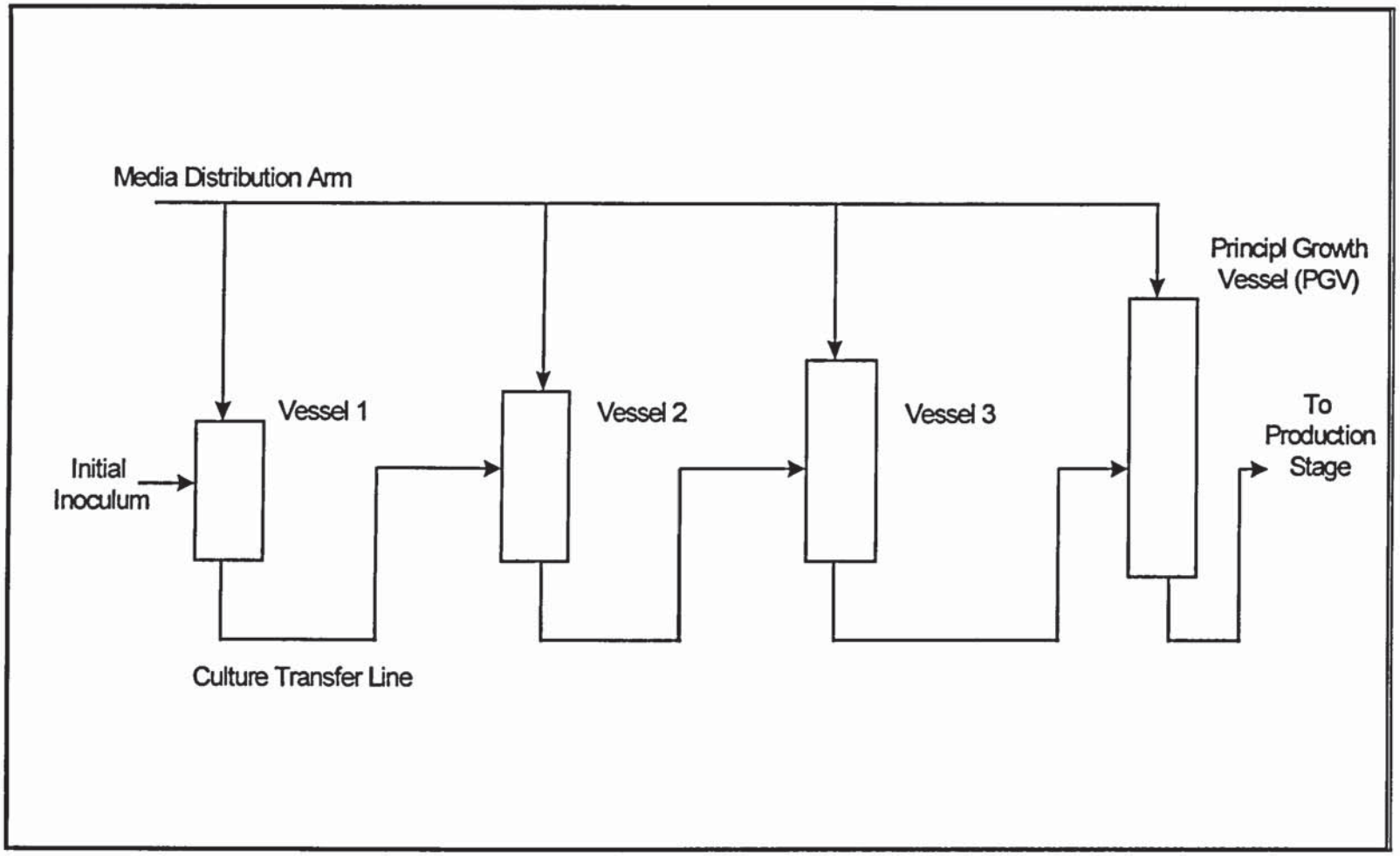

Figure 2.2 Process flowsheet diagram showing the train of progressively larger fermentation vessels up to and including the PGV.

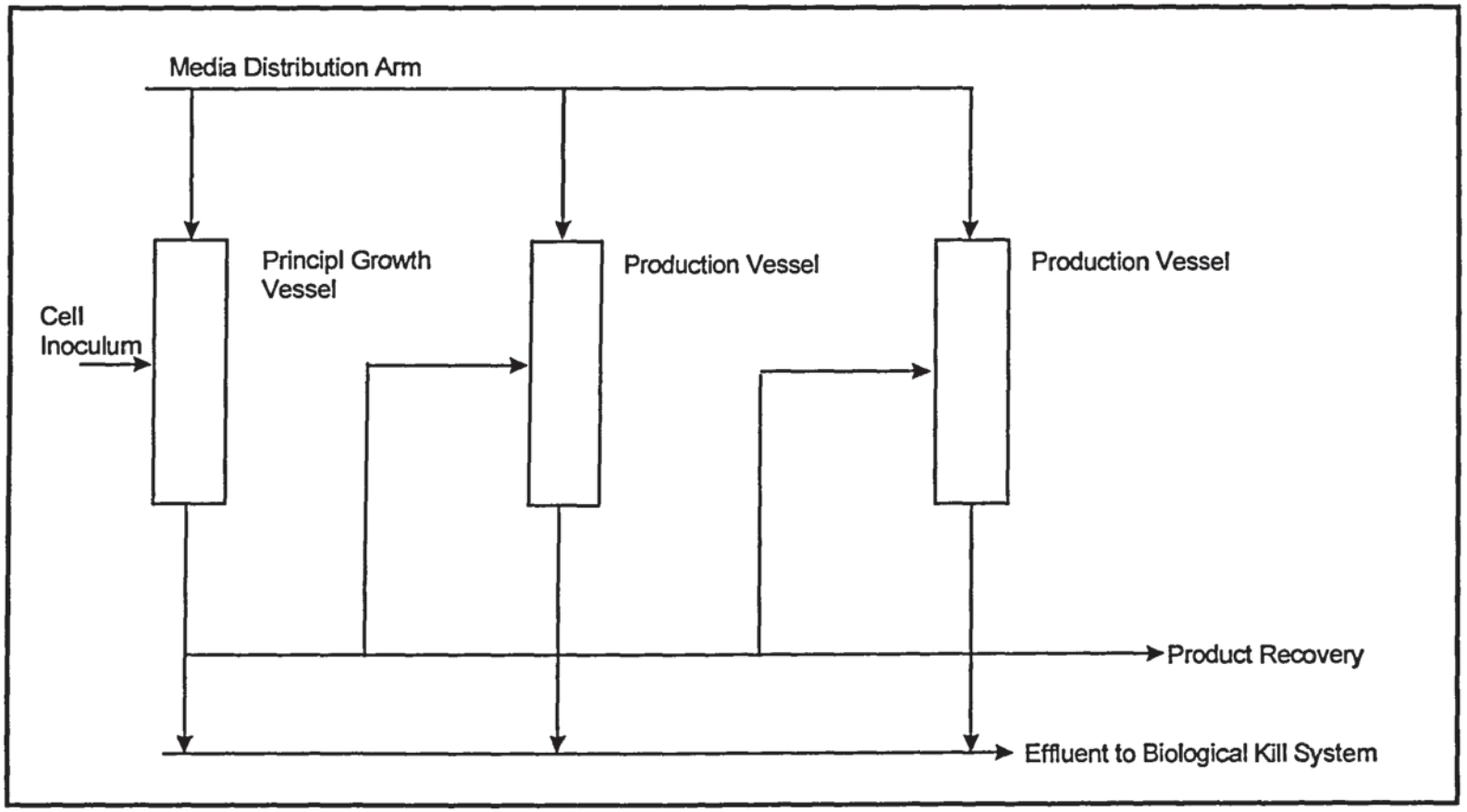

Figure 2.3 Process Flowsheet diagram showing the arrangement of the PGV and two production vessels that have been considered in the case studies presented in Chapter 2, together with their connections to harvesting and decontamination systems.

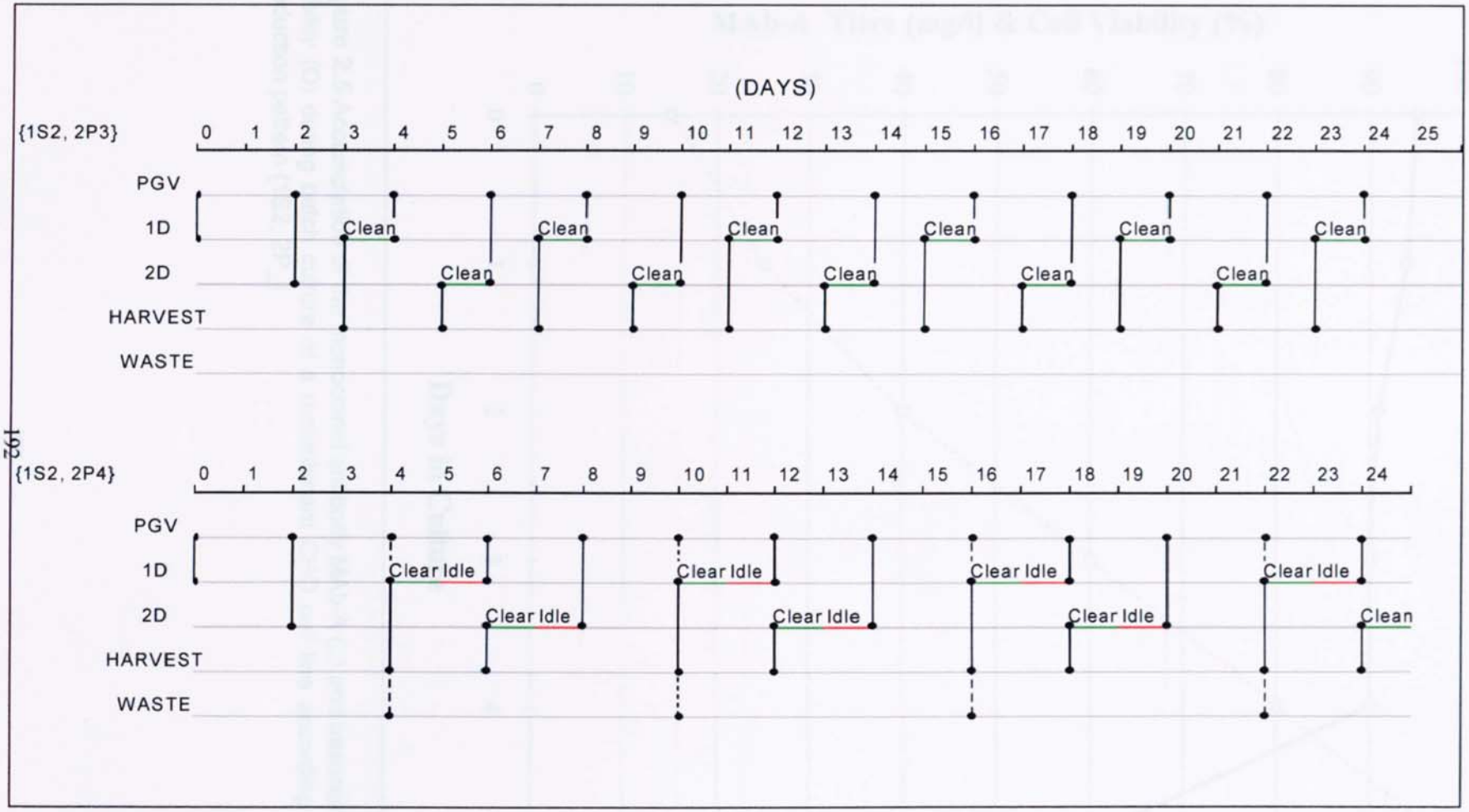

Figure 2.4 Network diagram that depicts the schedule of cell transfers between the PGV (S) and the production vessels (P_1 and P_2), and from the cell culture vessels to drain or product harvesting for the typical production patterns {1S2, 2P3} and {1S2, 2P4}.

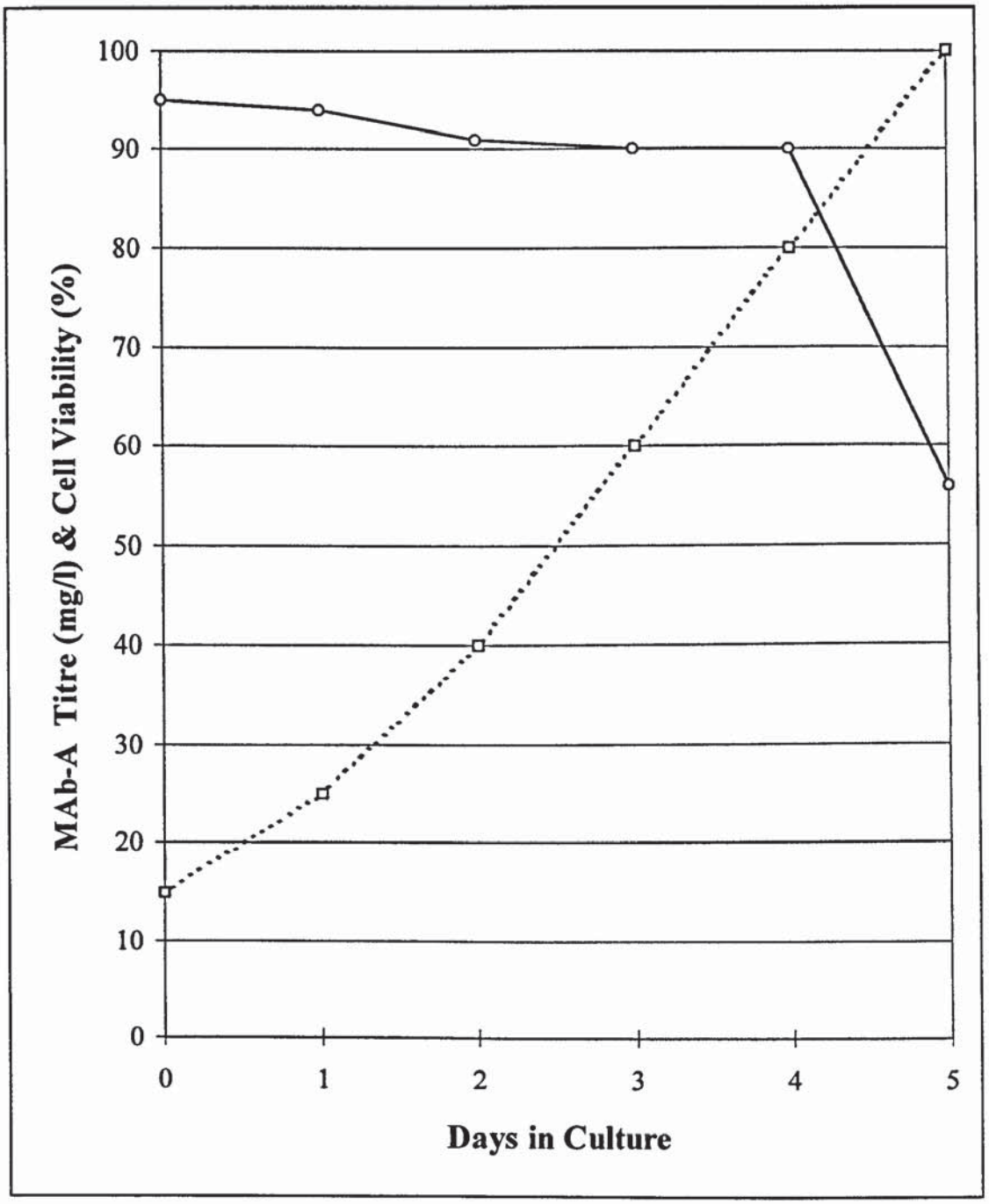

Figure 2.5 Accumulation of the monoclonal antibody MAb-A (□) and associated cell viability (O) during batch culture of a recombinant CHO cell line according to the production pattern {1S2, 2P_}

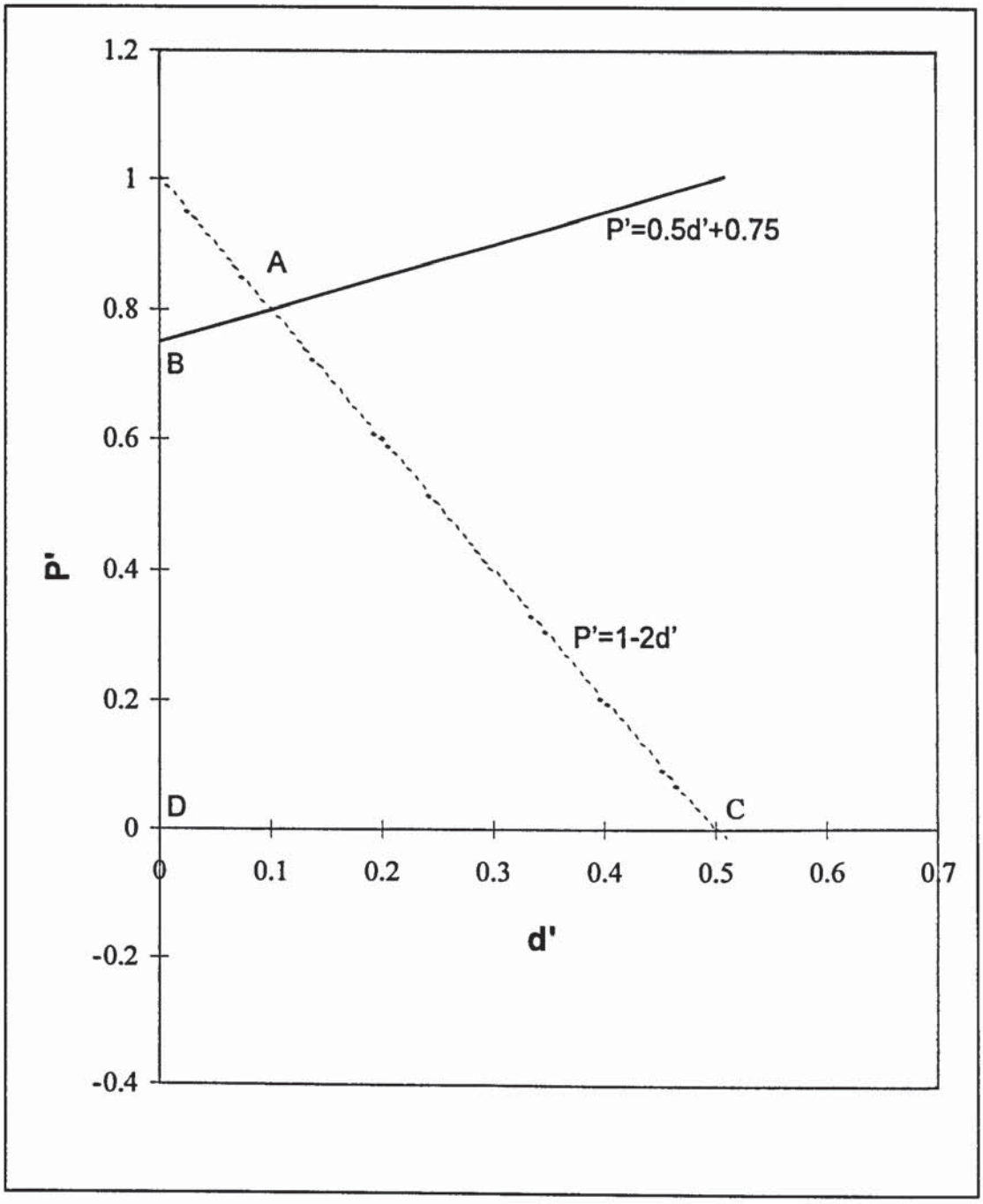

Figure 2.6 Feasible region (ABCD) in (P', d') space for the linear optimisation of Y' for the class of production patterns {1S2, 2P_}, employing the economic parameters of Table 2.1 .

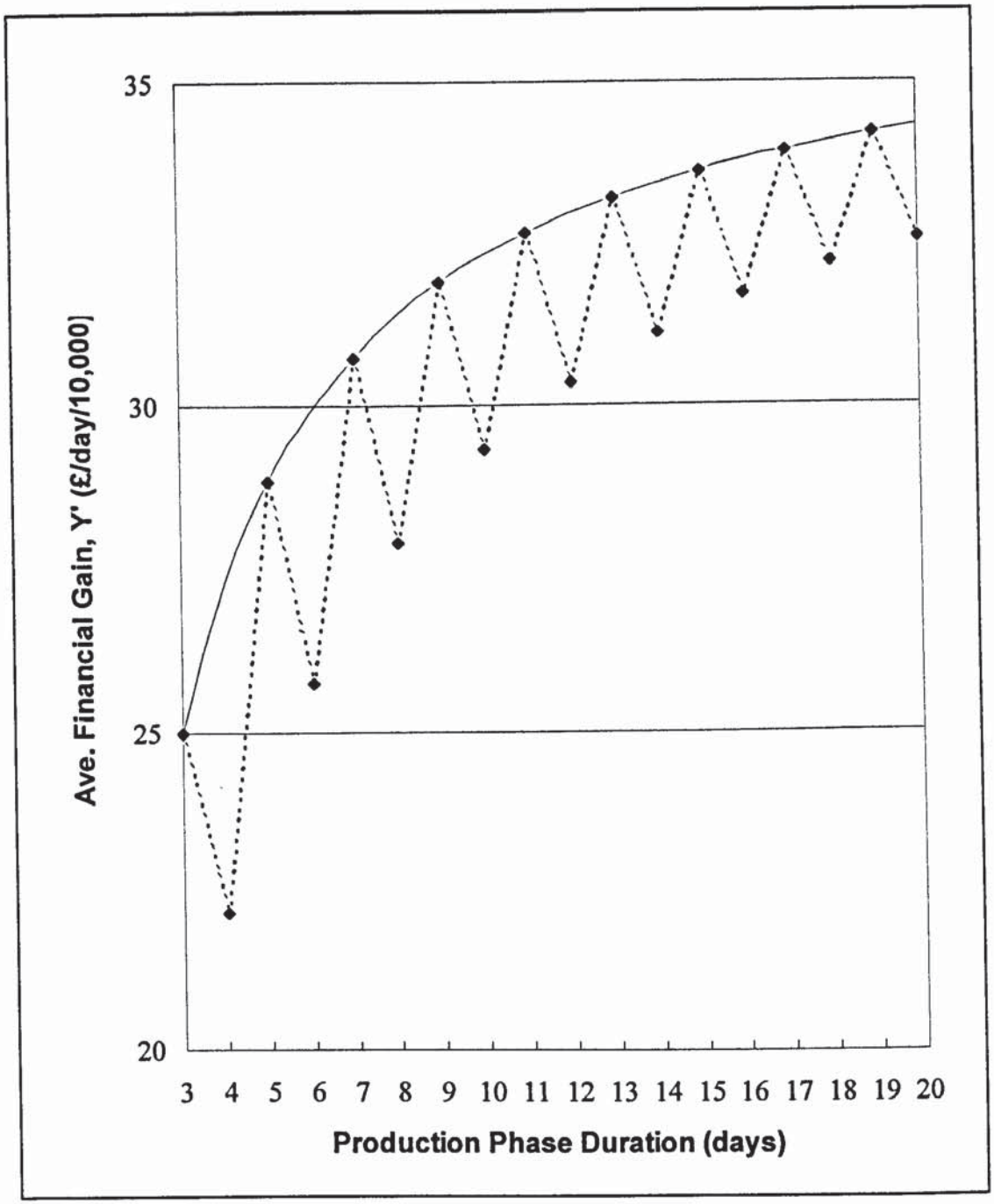

Figure 2.7 The variation of Y' for continuous (——) and permissible, discrete (—) values of P with the production patterns {1S2, 2P_}, indicating the asymptotic trend as $P \rightarrow \infty$.

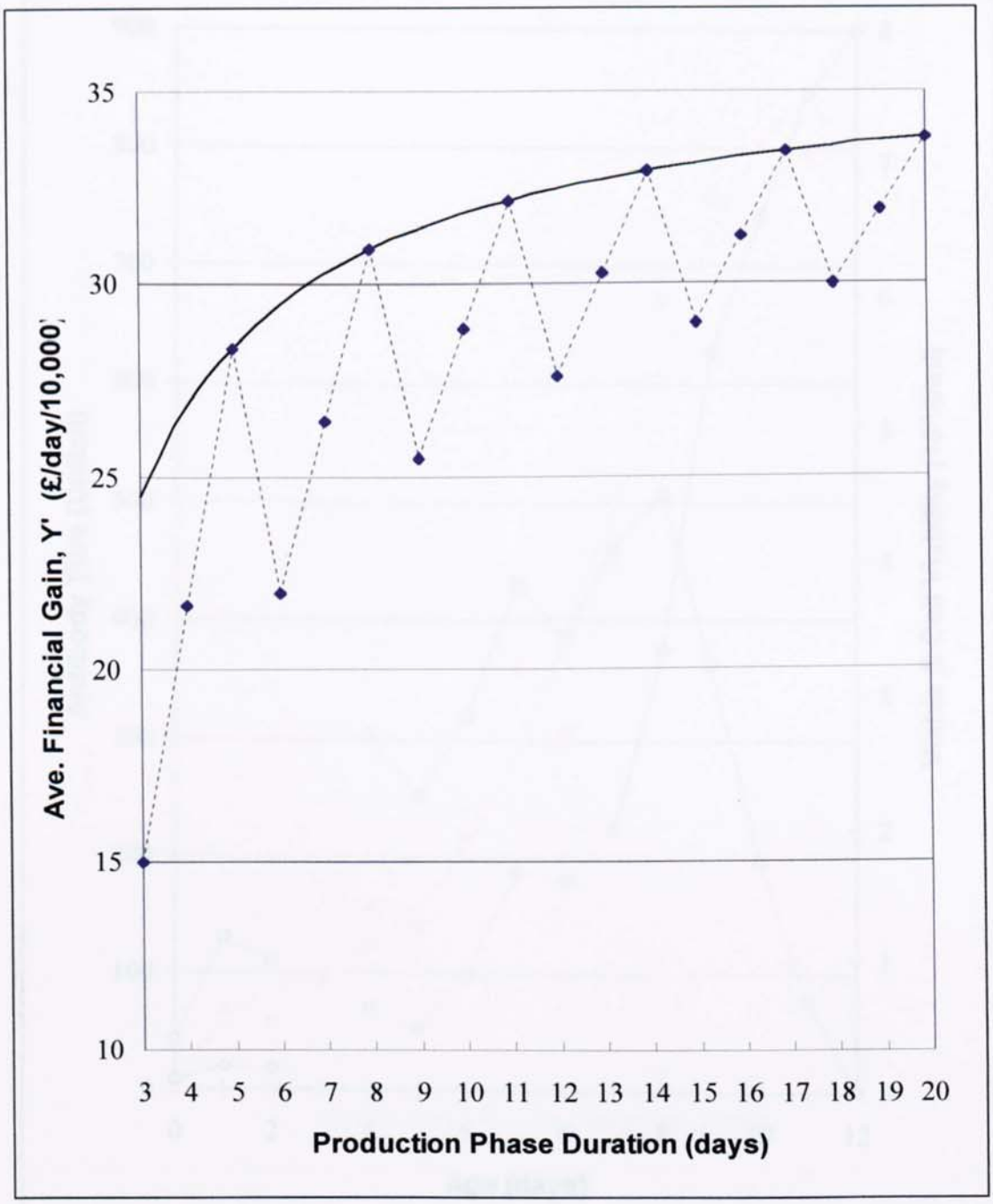

Figure 2.8 The variation of Y' for continuous (——) and permissible, discrete (---) values of P with the production patterns {1S3, 2P_}, indicating the asymptotic trend as $P \rightarrow \infty$.

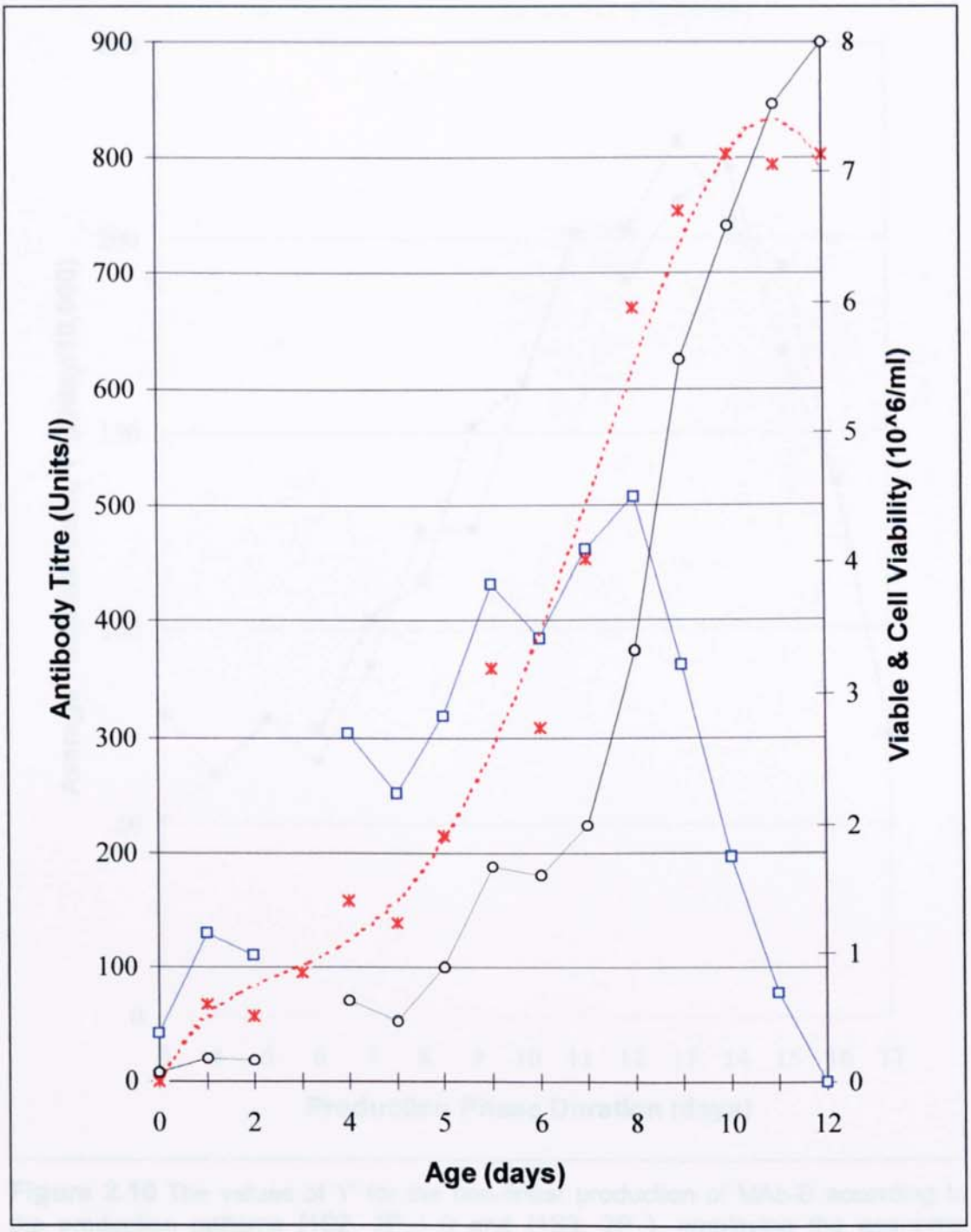

Figure 2.9 Accumulation of the monoclonal antibody MAb-B (*), viable cell density (□) and non-viable cell density (O) during extended culture of a recombinant NSO cell line according to the pattern {1S2, 2P_}. The trend of the fourth-order polynomial least-squares fit to the MAb-B titre variation is also indicated (----).

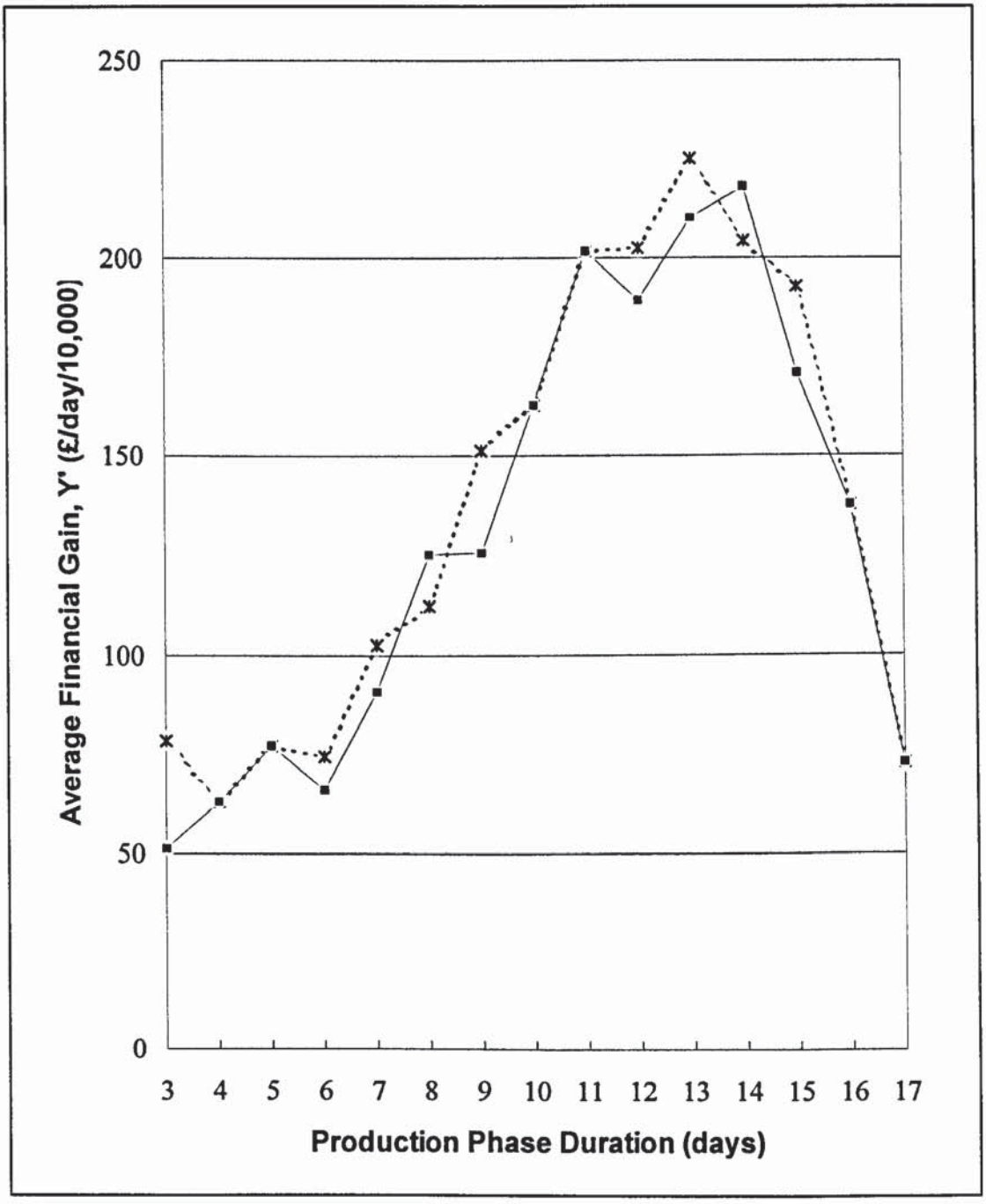

Figure 2.10 The values of Y' for the non-linear production of MAb-B according to the production patterns {1S2, 2P_} () and {1S3, 2P_}, employing the economic parameters of Table 2.1

APPENDIX B (Chapter III Figures)

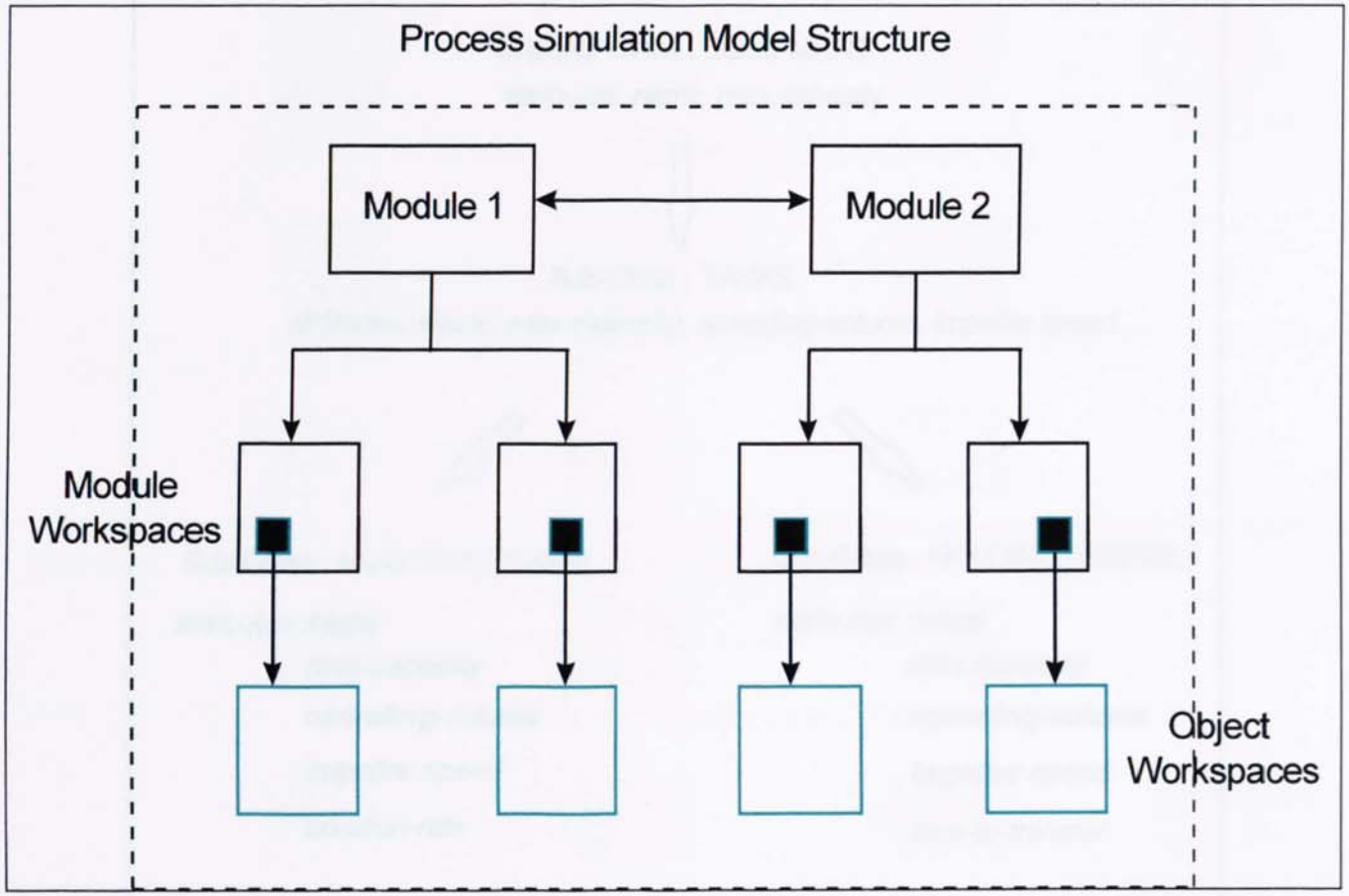

Figure 3.1 A schematic representation of the structural organisation of a hypothetical simulation model consisting of two modules. Each module has two workspaces. Each workspace holds a single object. Each object also has a workspace associated with it.

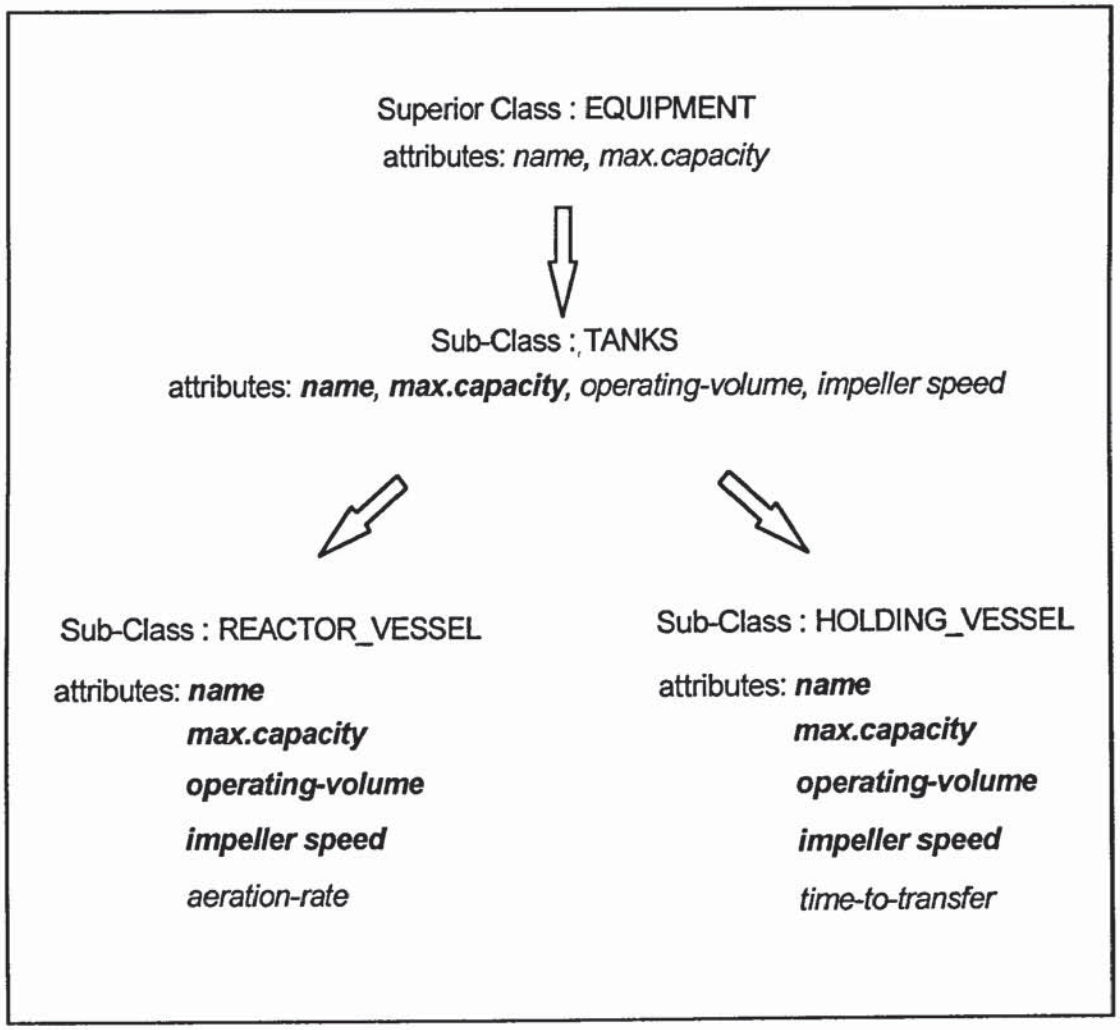

Figure.3.2 The idea of classification of objects into ordered classes and the inheritance of common attributes(parameters and variables).

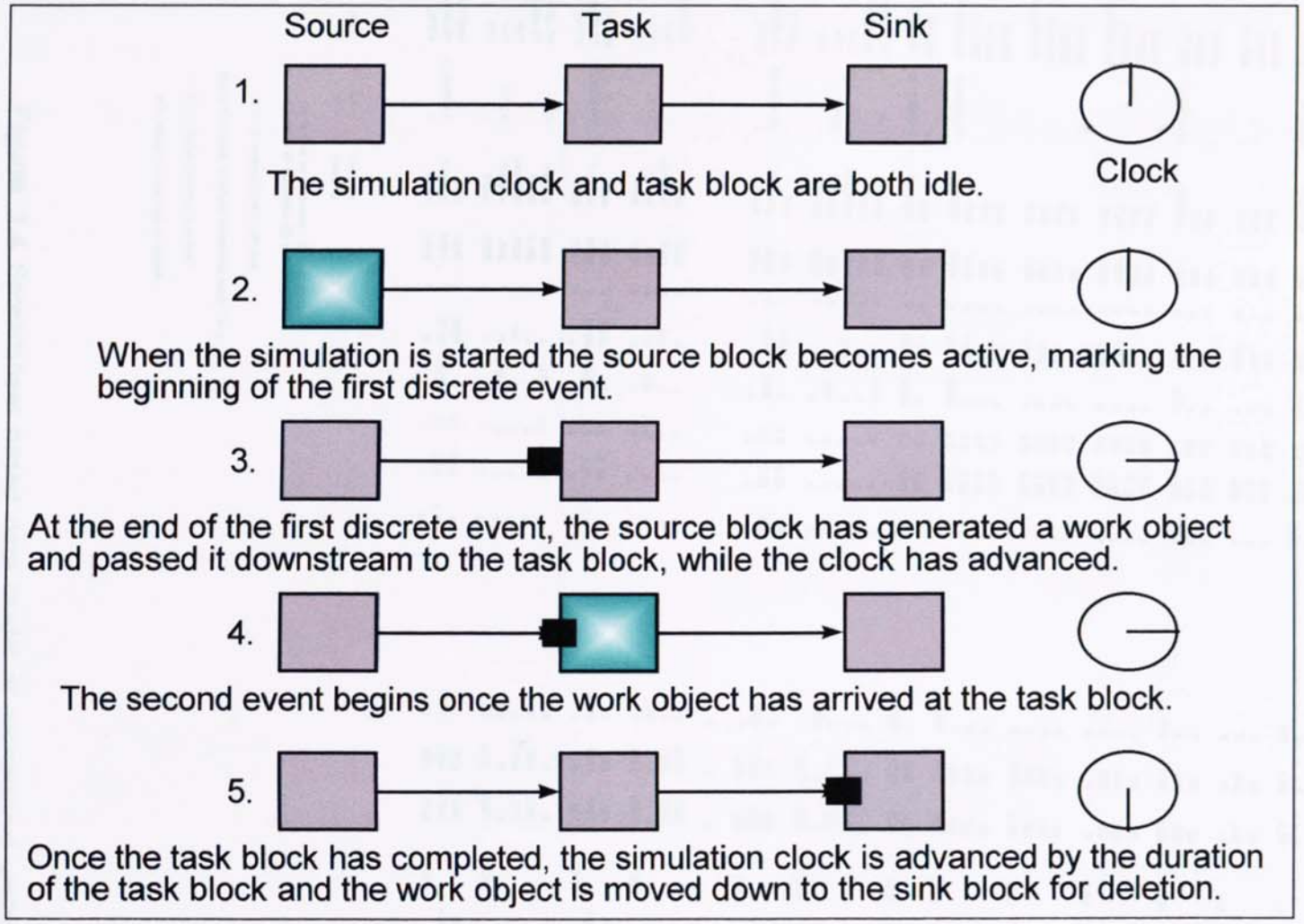

Figure 3.3 A simple ReThink™ process model consisting of three step illustrating the discrete event simulation paradigm (Adapted from [3]).

APPENDIX B

CCC C1H PRODUCTION. VESSEL SCHEDULE

Day	Vessel 1	Vessel 2	Operation	V1-	V2-	V1+	V2+	X1-	X1+	X2+	Vtrans	V1D	VDtotal	Drainage	M1	M2	Mtot	Media
28	CV30	CV100	Transfer	25	0	15	42	0 8	0.35	0 35	18	0.06	0.06	0 06	8	24	32	32
28	ne in BUC>																	
	CV100	-	Sub	42	0	95	0	0 8	0.35	0	0	0.44	0.44	0.44	53	0	53	53
30	CV100	CV600	Transfer	95	0	20	200	0 8	0 35	0 35	88	(1.25)	(1.25)	(1 25)	11	113	124	124
32	CV100	-	Sub	20	0	50	0	0 8	0.35	0	0	(1.88)	(1 88)		28	0	28	
	CV600	-	Sub	200	0	455	0	0 8	0 35	0	0	0 94	0.94	(0 94)	256	0	256	284
34	CV100	-	Sub	50	0	100	0	0.8	0.35	0	0	6	6		56	0	56	
	CV600	CV1250	Transfer	450	0	600	425	0 8	0.35	0.35	188	1.56	1.56	8	338	239	577	633
36	CV100	-	Sub	100	0	100	0	0 8	0.35	0	0	56	56		56	0	56	
	CV600	-	Sub	600	0	600	0	0.8	0 35	0	0	338	338		338	0	338	
	CV1250	-	Sub	450	0	1000	0	0.8	0 35	0	0	13	13	406	563	0	563	956
38	CV100	-	Sub	100	0	100	0	0 8	0.35	0	0	56	56		56	0	56	
	CV600	-	Sub	600	0	600	0	0 8	0 35	0	0	338	338		338	0	338	
	CV1250	CV8000.S1	Transfer	1000	0	1000	2000	0.8	0.275	0.26	650	6.25	6.25	400	656	1350	2006	2400
40	CV100	-	Sub	100	0	100	0	0 8	0.35	0	0	56	56		56	0	56	
	CV600	-	Sub	600	0	600	0	0 8	0 35	0	0	338	338		338	0	338	
	CV1250	-	Sub	1000	0	1000	0	0 8	0 35	0	0	563	563		563	0	563	
	CV8000.S1	-	Sub	2000	0	4550	0	0.8	0.35	0	0	9	9	968	2559	0	2559	3518
42	CV100	-	Sub	100	0	100	0	0.8	0.35	0	0	56	56		56	0	56	
	CV600	-	Sub	600	0	600	0	0.8	0.35	0	0	338	338		338	0	338	
	CV1250	-	Sub	1000	0	1000	0	0 8	0.35	0	0	563	563		563	0	563	
	CV8000.S1	-	Sub	4900	0	8000	0	0 8	0.35	0	0	1400	1400	2356	4500	0	4500	5456
44	CV100	-	Sub	100	0	100	0	0 8	0.35	0	0	56	56		56	0	56	
	CV600	-	Sub	600	0	600	0	0.8	0 35	0	0	338	338		338	0	338	
	CV1250	-	Sub	1000	0	1000	0	0 8	0.35	0	0	563	563		563	0	563	
	CV8000.S1	CV8000.P1	Transfer	8000	0	8000	8000	0 8	0.35	0.3	3000	1500	1500	2456	4500	5000	9500	10456
46	CV1250	-	Sub	1000	0	1000	0	0 8	0.35	0	0	563	563		563	0	563	
	CV8000.S1	CV8000.P2	Transfer	8000	0	8000	8000	0 8	0.35	0.3	3000	1500	1500	2063	4500	5000	9500	10063
47	CV8000 P1	HV1	Harvest	8000	0	0	8000	0 8	0	0	0	0	0		0	0	0	
	CV8000.P1	-	Wash	8000	0	0	0	0	0	0	0	2000	2000		0	0	0	
	HV1	-	Ultrafilter	8000	0	800	0	0	0	0	0	7200	7200		0	0	0	
	HV1	UFCV	Transfer	800	0	0	800	0	0	0	800	0	0		0	0	0	
	HV1	-	Wash	8000	0	0	0	0	0	0	0	2000	2000	11200	0	0	0	0
48	CV1250	-	Sub	1000	0	1000	0	0 8	0 35	0	0	563	563		563	0	563	
	CV8000.S1	CV8000 P1	Transfer	8000	0	8000	8000	0.8	0.35	0 3	3000	1500	1500		4500	5000	9500	
	UFCV	-	Wash	2000	0	0	0	0	0	0	0	500	500	2563	0	0	0	10063
											0	0	0		0	0	0	
											0	2000	2000		0	0	0	
	CV8000 P2	-	Wash	8000	0	0	0	0	0	0	0	7200	7200		0	0	0	
	HV1	-	Ultrafilter	8000	0	800	0	0	0	0	800	0	0		0	0	0	
	HV1	UFCV	Transfer	800	0	0	800	0	0	0	0	2000	2000	11200	0	0	0	0
	HV1	-	Wash	8000	0	0	0	0	0	0								
											0	563	563		563	0	563	
50	CV1250	-	Sub	1000	0	1000	0	0.8	0.35	0	3000	1500	1500		4500	5000	9500	
	CV8000.S1	CV8000.P2	Transfer	8000	0	8000	8000	0.8	0.35	0.3	0	500	500	2563	0	0	0	10063
	UFCV	-	Wash	2000	0	0	0	0	0	0								
											0	0	0		0	0	0	
51	CV8000.P1	HV1	Harvest	8000	0	0	8000	0 8	0	0	0	2000	2000		0	0	0	
	CV8000.P1	-	Wash	8000	0	0	0	0	0	0	0	7200	7200		0	0	0	
	HV1	-	Ultrafilter	8000	0	800	0	0	0	0	800	0	0		0	0	0	
	HV1	UFCV	Transfer	800	0	0	800	0	0	0	0	2000	2000	11200	0	0	0	0
	HV1	-	Wash	8000	0	0	0	0	0	0								
											0	563	563		563	0	563	
52	CV1250	-	Sub	1000	0	1000	0	0.8	0.35	0	3000	1500	1500		4500	5000	9500	
	CV8000 S1	CV8000 P1	Transfer	8000	0	8000	8000	0.8	0.35	0.3	0	500	500	2563	0	0	0	10063
	UFCV	-	Wash	2000	0	0	0	0	0	0								

KEY:

V1 = Vessel 1
V2 = Vessel 2

- = Before an Operation (@ start of day)
+ = After an Operation (@ end of day)

X = Cell density (x10^6 cells/ml)

Vtrans = Volume of culture transferred from 1 to 2

V1D = Volume flow from 1 to drain

M = Nutrient media feed to vessels

Figure 3.4 Spreadsheet based data model of monoclonal antibody manufacturing facility.

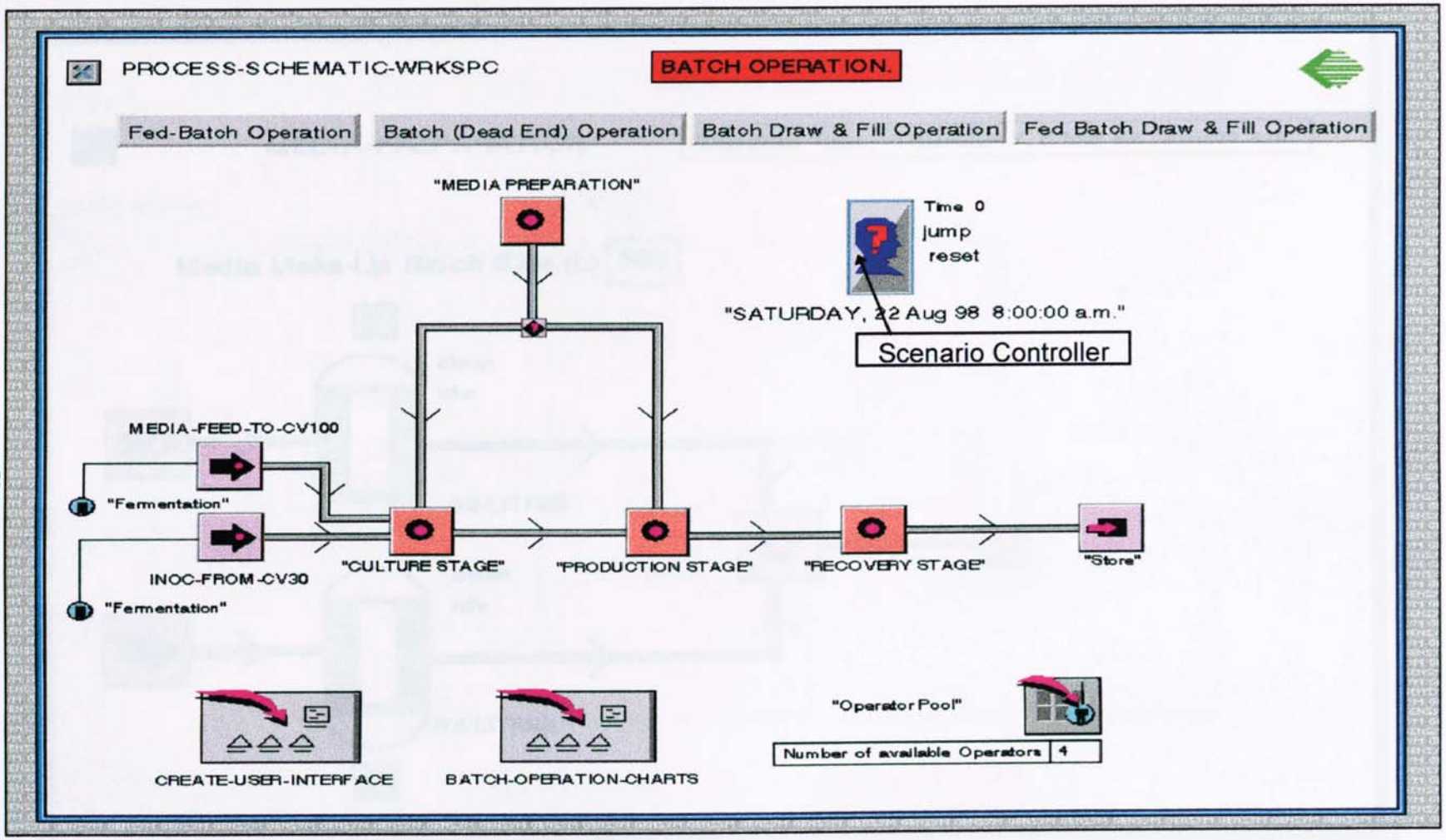

Figure 3.5 Simulation model representation of the overall process schematic. The four main process stages are shown as orange coloured blocks. This indicated that each block contains a further level of detail.

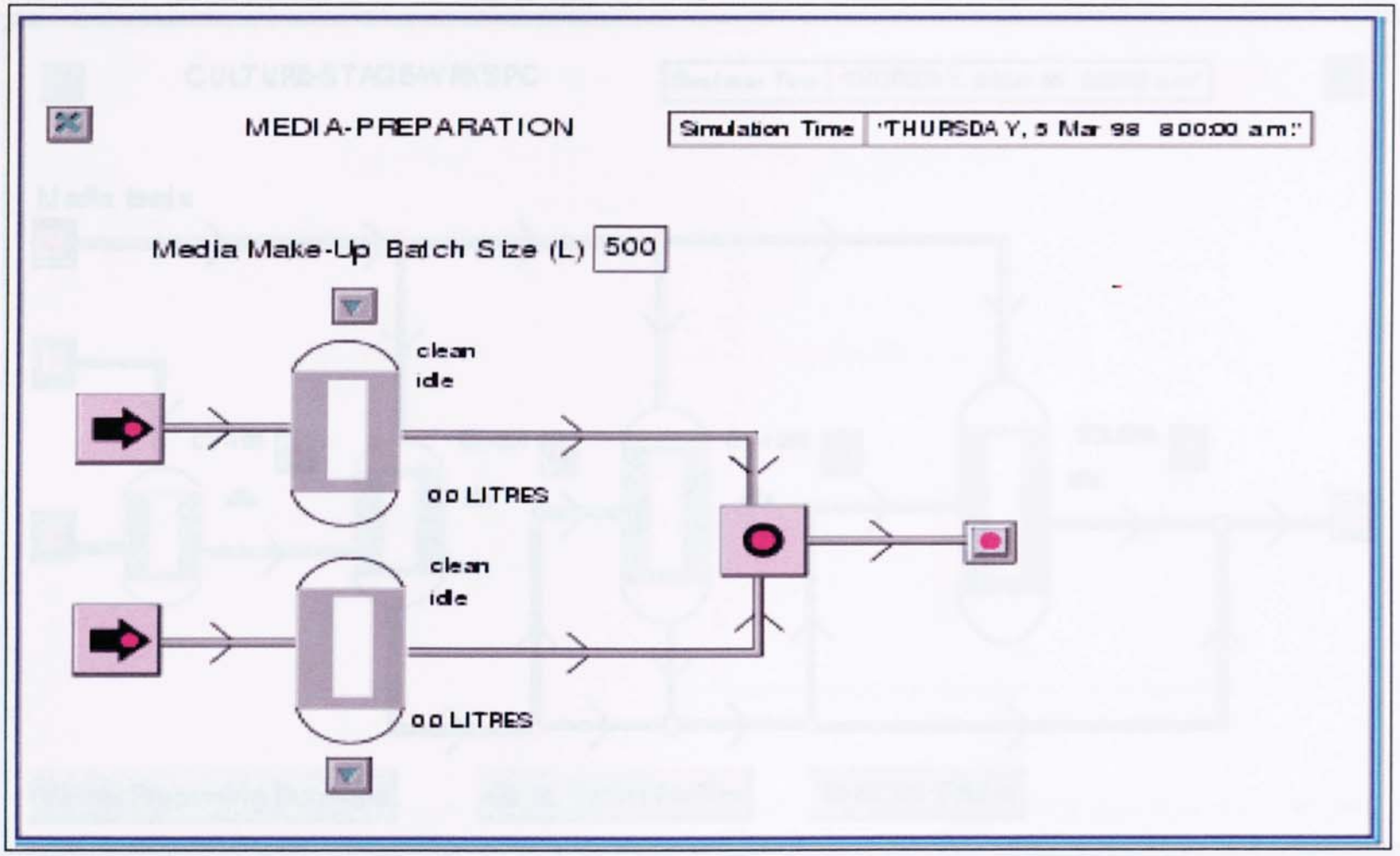

Figure 3.6 Screen shot showing the next level of process detail for the Media Preparation Stage. Two media blend vessels are shown each connecting into the media filter unit.

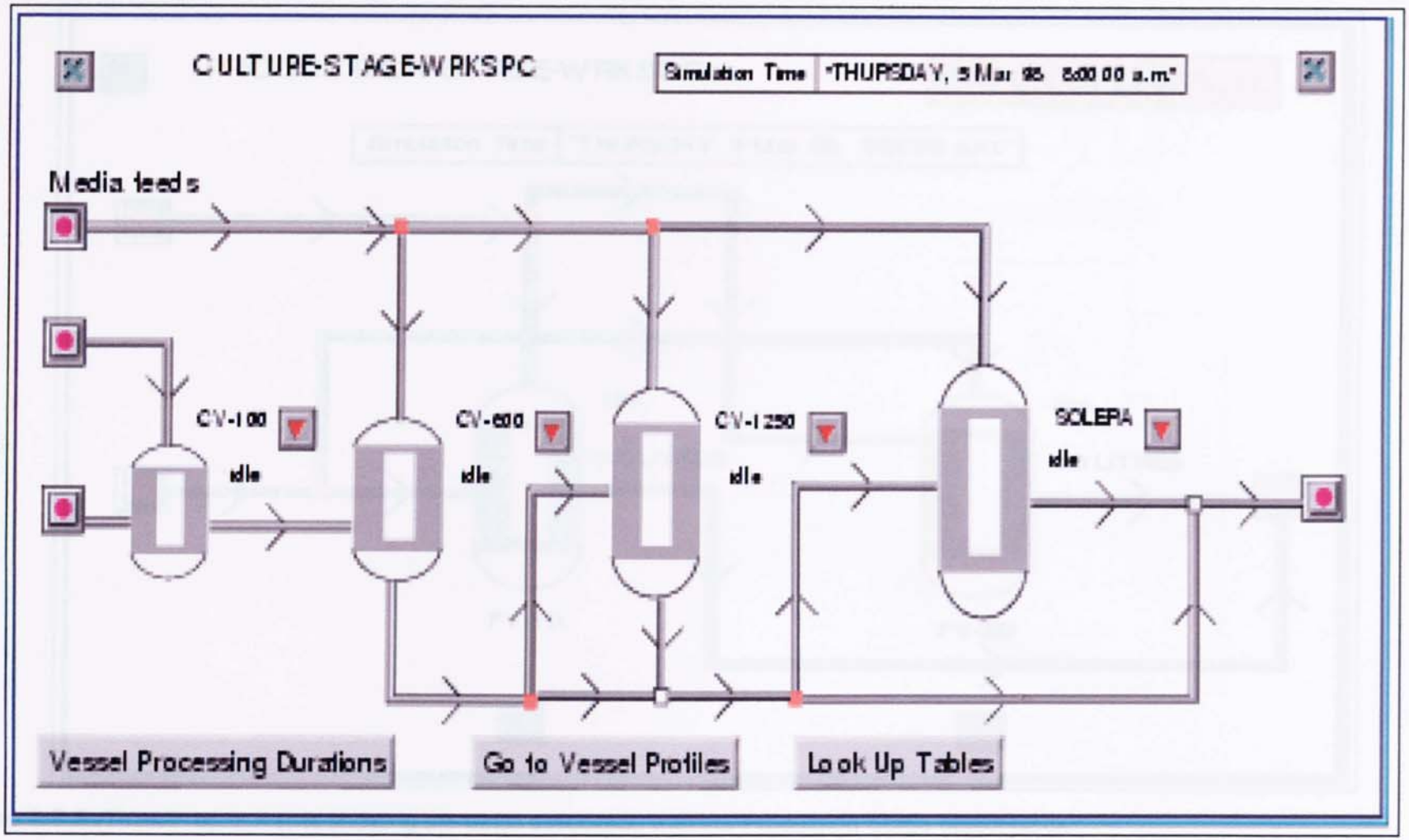

Figure 3.7 ReThink™ screen shot showing the detail associated with the Culture Stage object block.

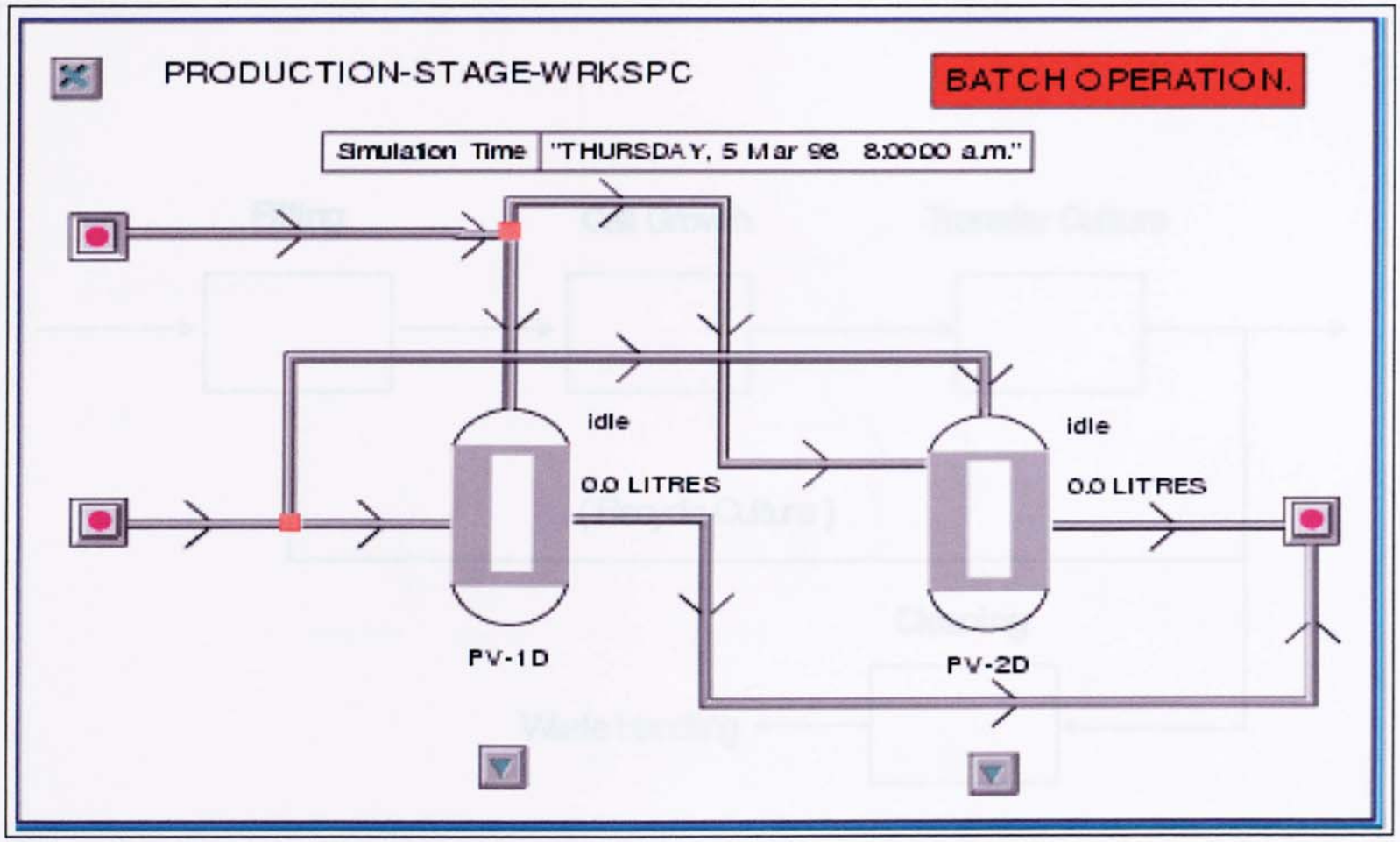

Figure 3.8 ReThink™ screen shot showing the detail associated with the Production Stage object block.

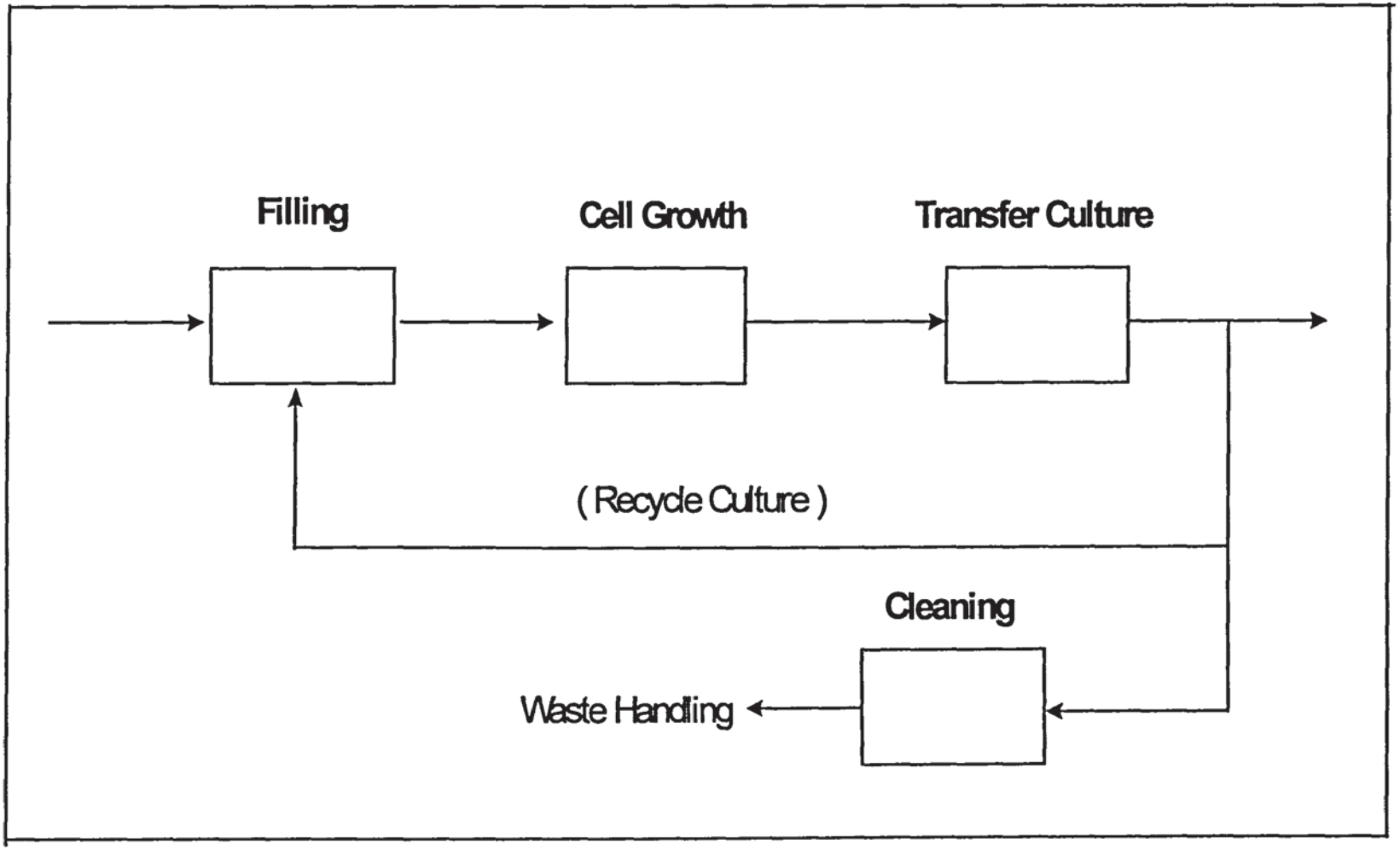

Figure 3.9 A generalised Activity-on-Node diagram showing the process logic underlying the operation of a culture vessel.

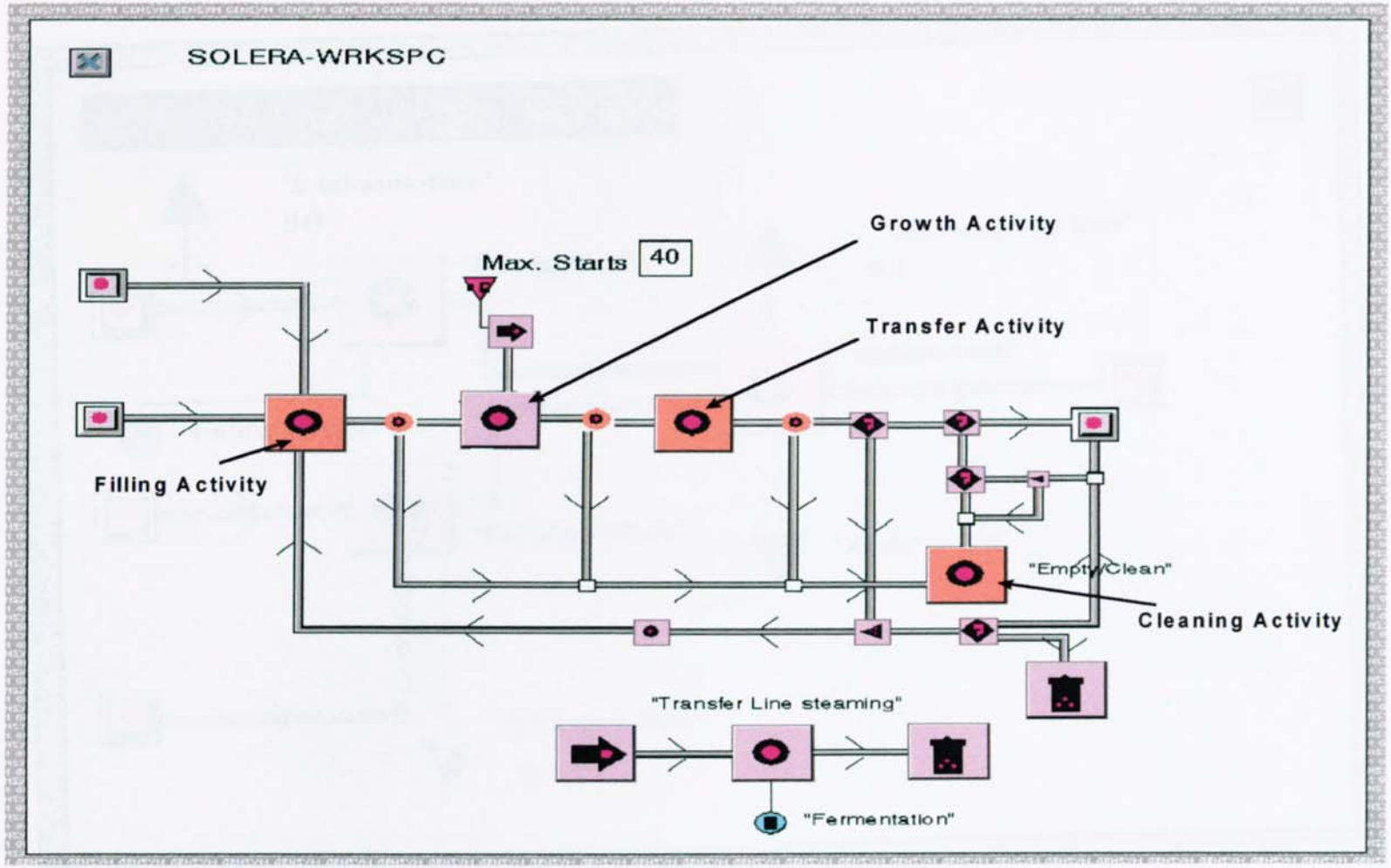

Figure 3.10 A ReThink™ process activity network diagram showing the underlying activities that compose the operation of a culture vessel.

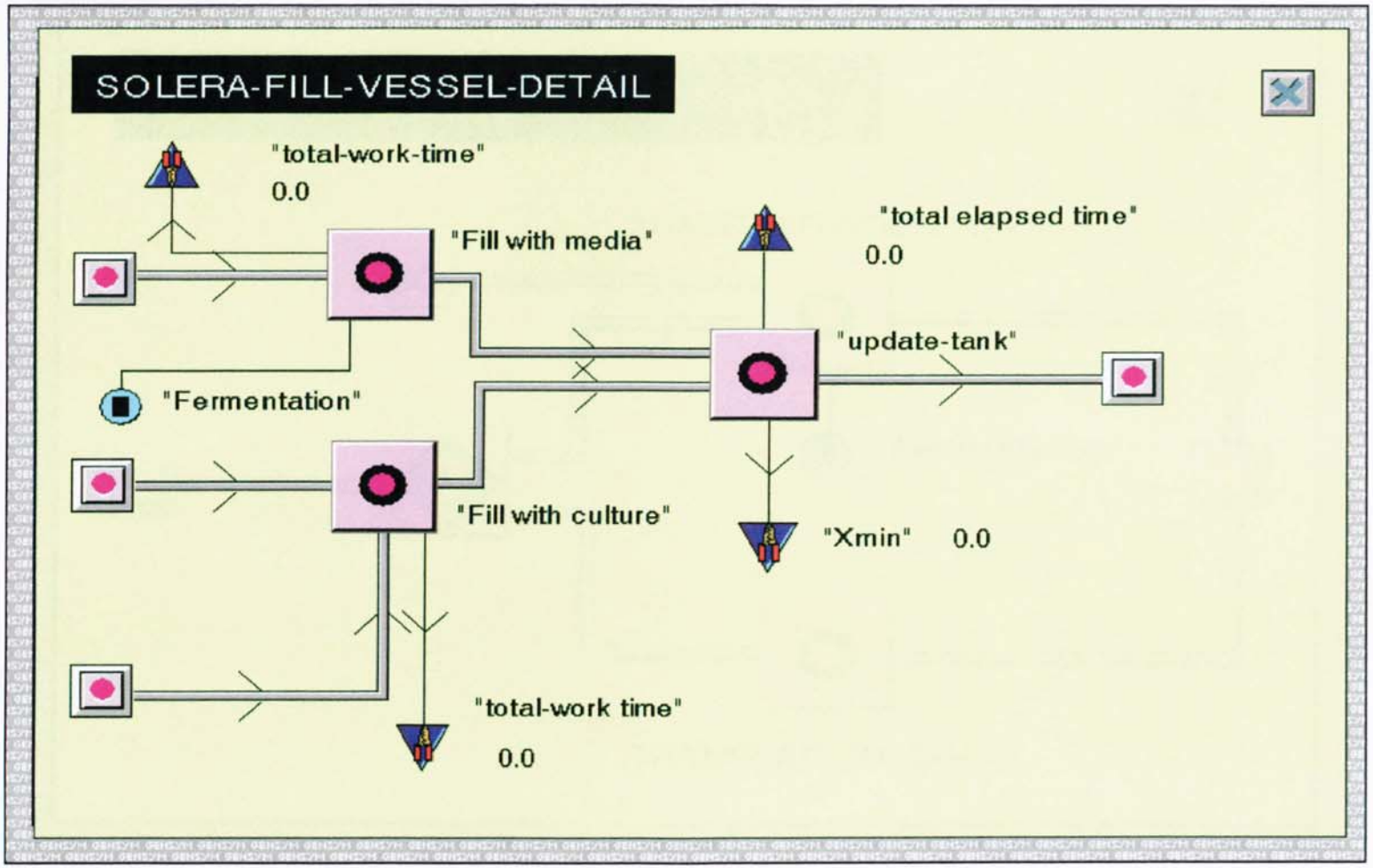

Figure 3.11 ReThink™ process activity network screen shot showing the logic structure of the three 'sub-activities' that comprise the vessel **Filling** activity.

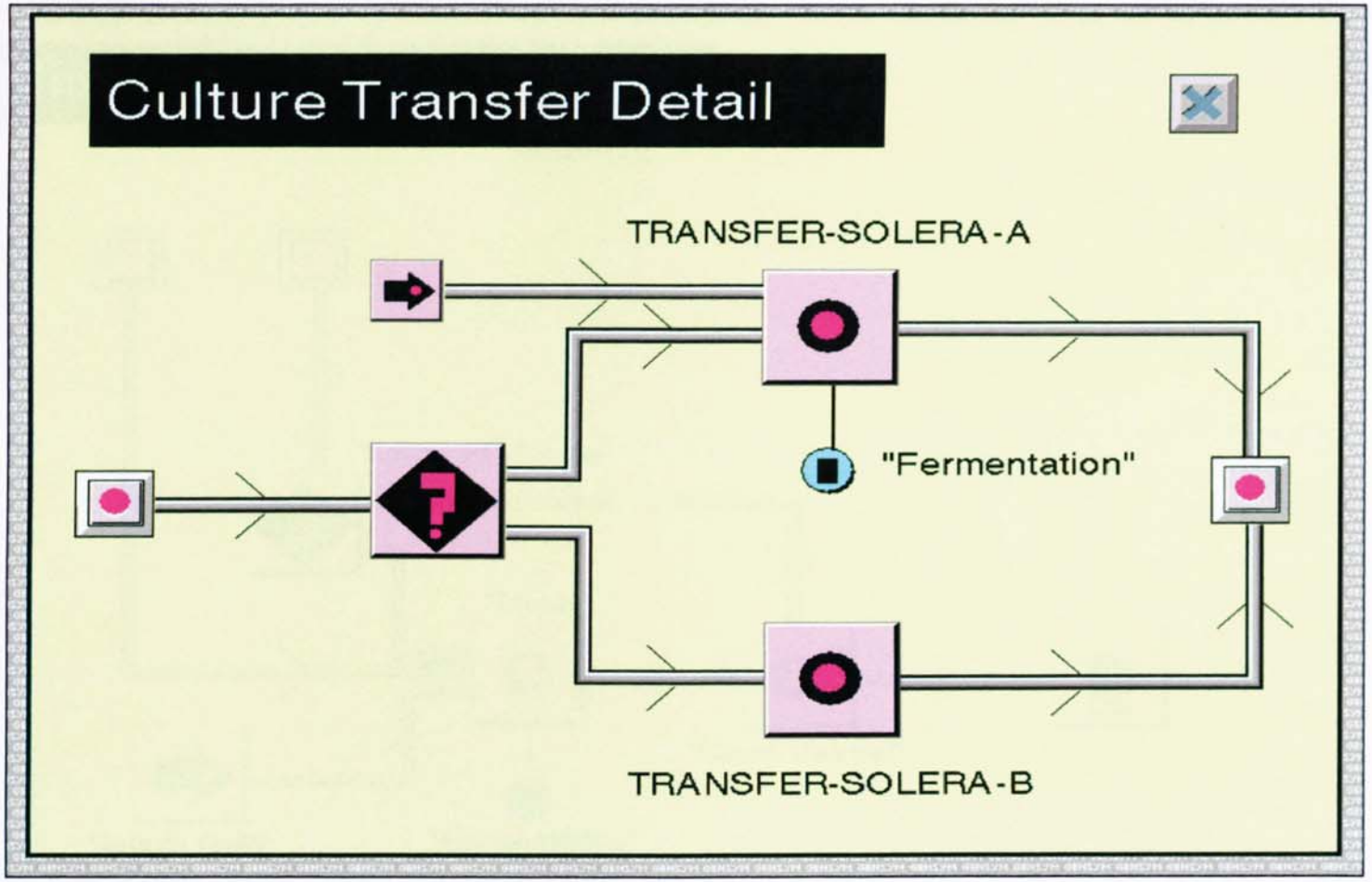

Figure 3.12 Process model representation of the 'sub-activities' network diagram associated with the culture **Transfer** activity.

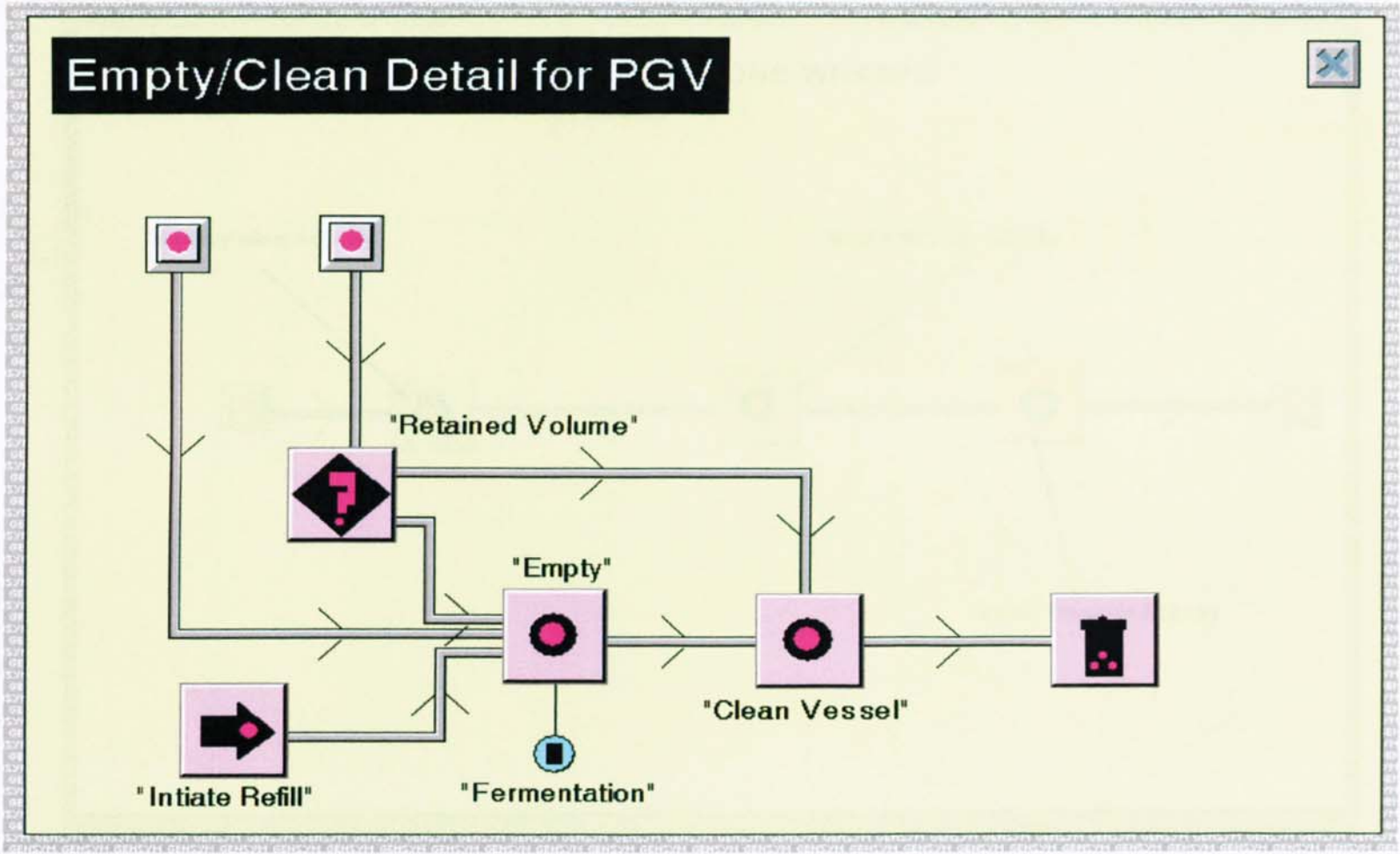

Figure 3.13 Process model representation of the 'sub-activities' network diagram associated with the emptying and or cleaning of a culture or production vessel.

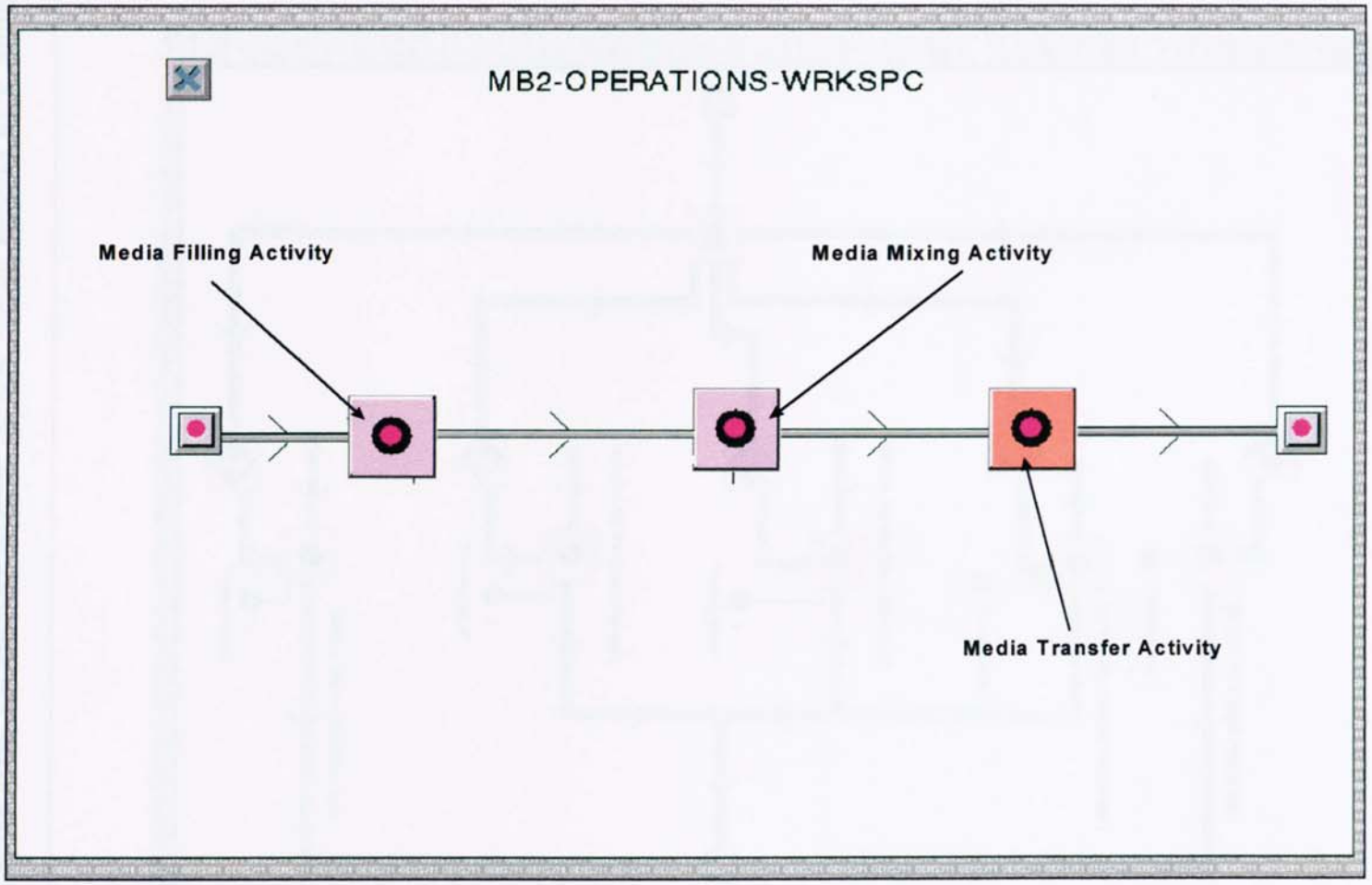

Figure 3.14 The process activity network diagram describing the underlying activity logic associated with the operation of a media blend vessel in the media preparation stage of the process.

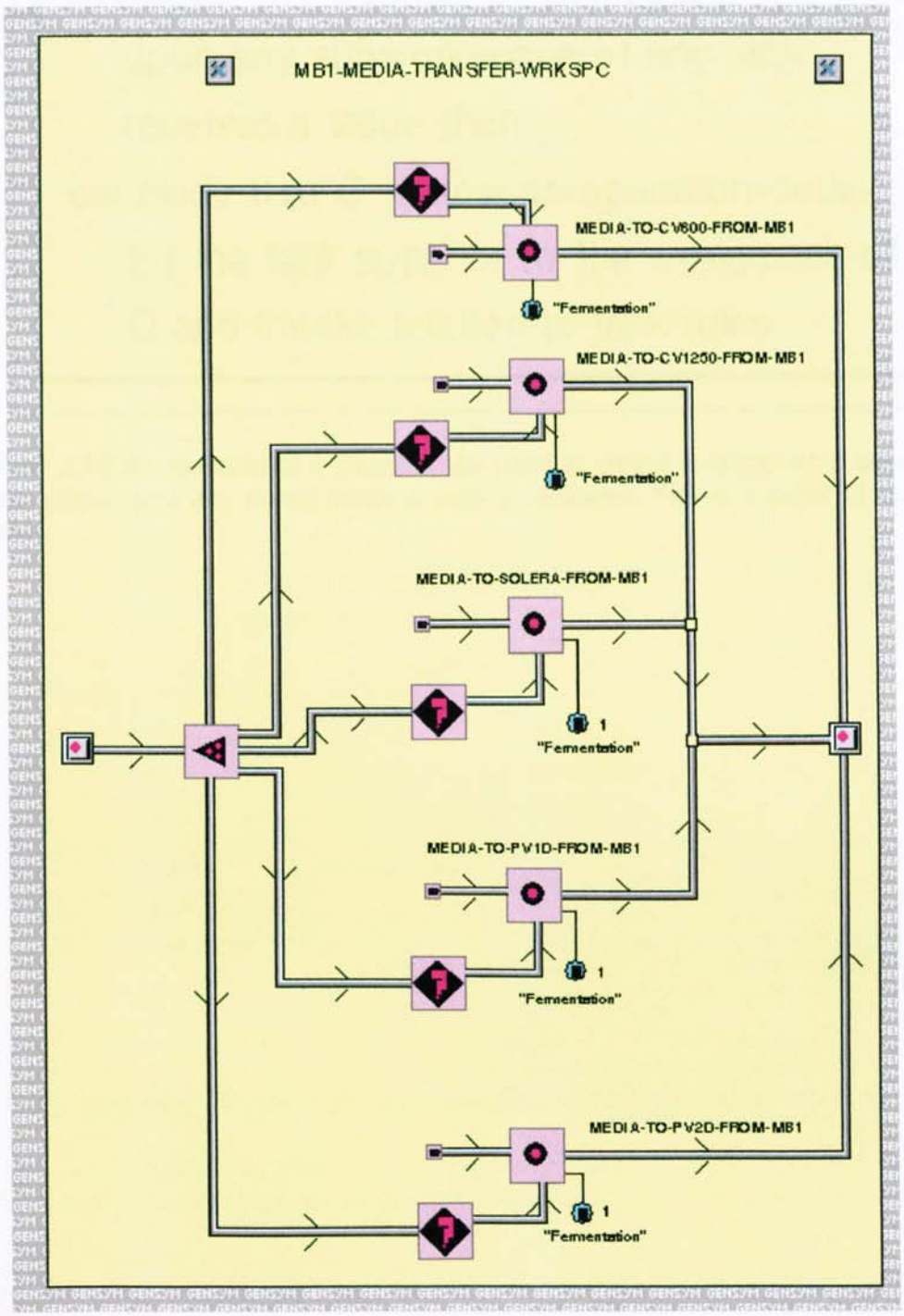

Figure 3.15 Process activity network diagram showing the detail associated with the media **Transfer** activity shown in Figure 3.14.

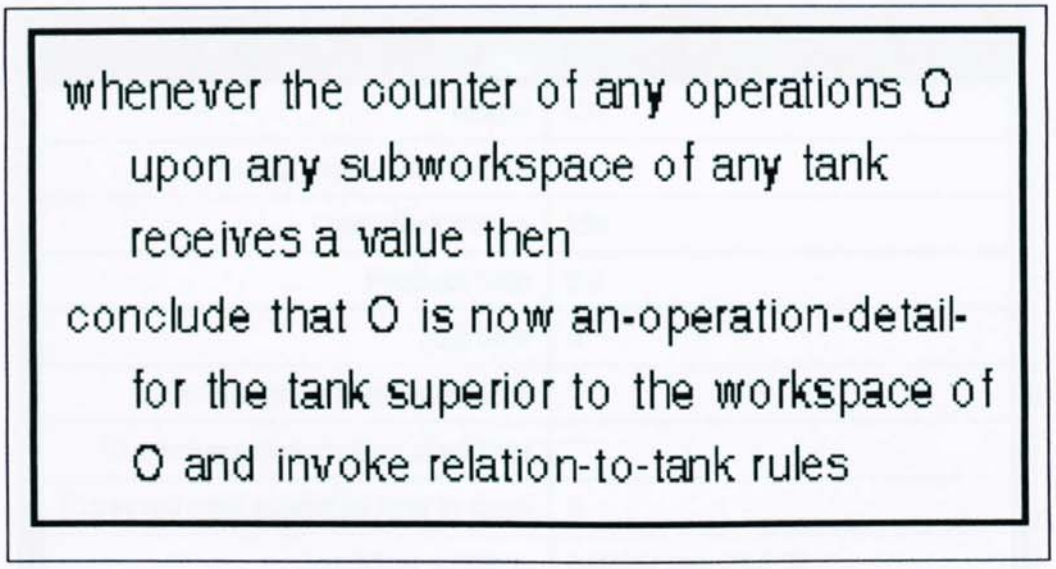

Figure 3.16 An example of a Design Rule used to create a relationship between any vessel (Tank) and any object block activity (operations) that is a detail of that vessel (Tank).

SOLERA, a tank	
Notes	OK
Contamination status	clean
Operating status	idle
Product type	0.0
Idle time	0
Initial equipment item start time	""
Current equipment item start time	""
Expected next available time in days	0
Operating strategy	batch-draw-and-fill
Current culture age in days	0
Max capacity	9000 LITRES
Batches generated	0
Contaminated batches	0
Fresh media volume	0.0 LITRES
Total media processed	0.0
Culture volume	0.0 LITRES
Total operating volume	0.0 LITRES
Waste volume	0.0 LITRES
Product concentration at end	0 UNITS-PER-LITRE
Productivity	0.0 UNITS-PER-LITRE-PER-DAY
Cost per unit product	0.0 POUNDS
Costs	an operations-cost-subtable

Figure 3.17 An attribute table listing the process parameters and values that a user of the simulation model would see associated with the PGV in this case.

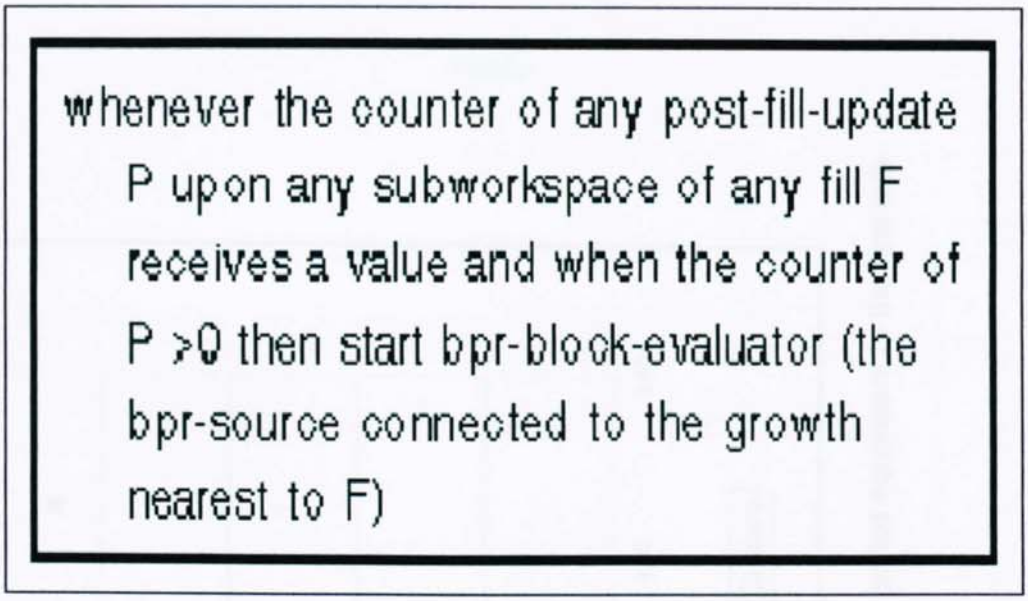

Figure 3.18 An example of a design rule used to trigger the Source block connected to every Growth activity of every culture and production vessel.

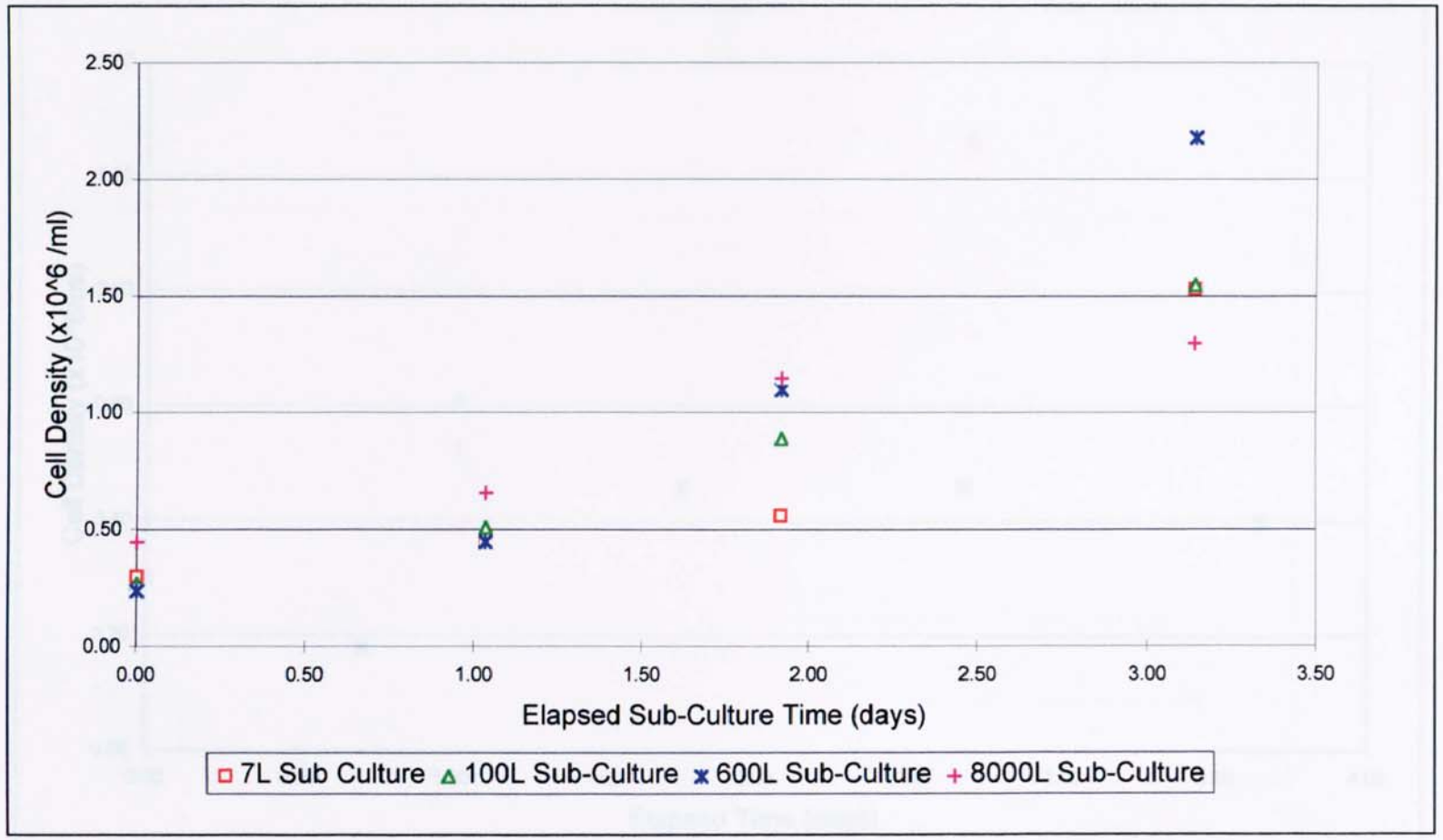

Figure 3.19 A plot of the cell densities achieved by four culture stage vessels over an operating period equivalent to the first sub-cultures of these vessels.

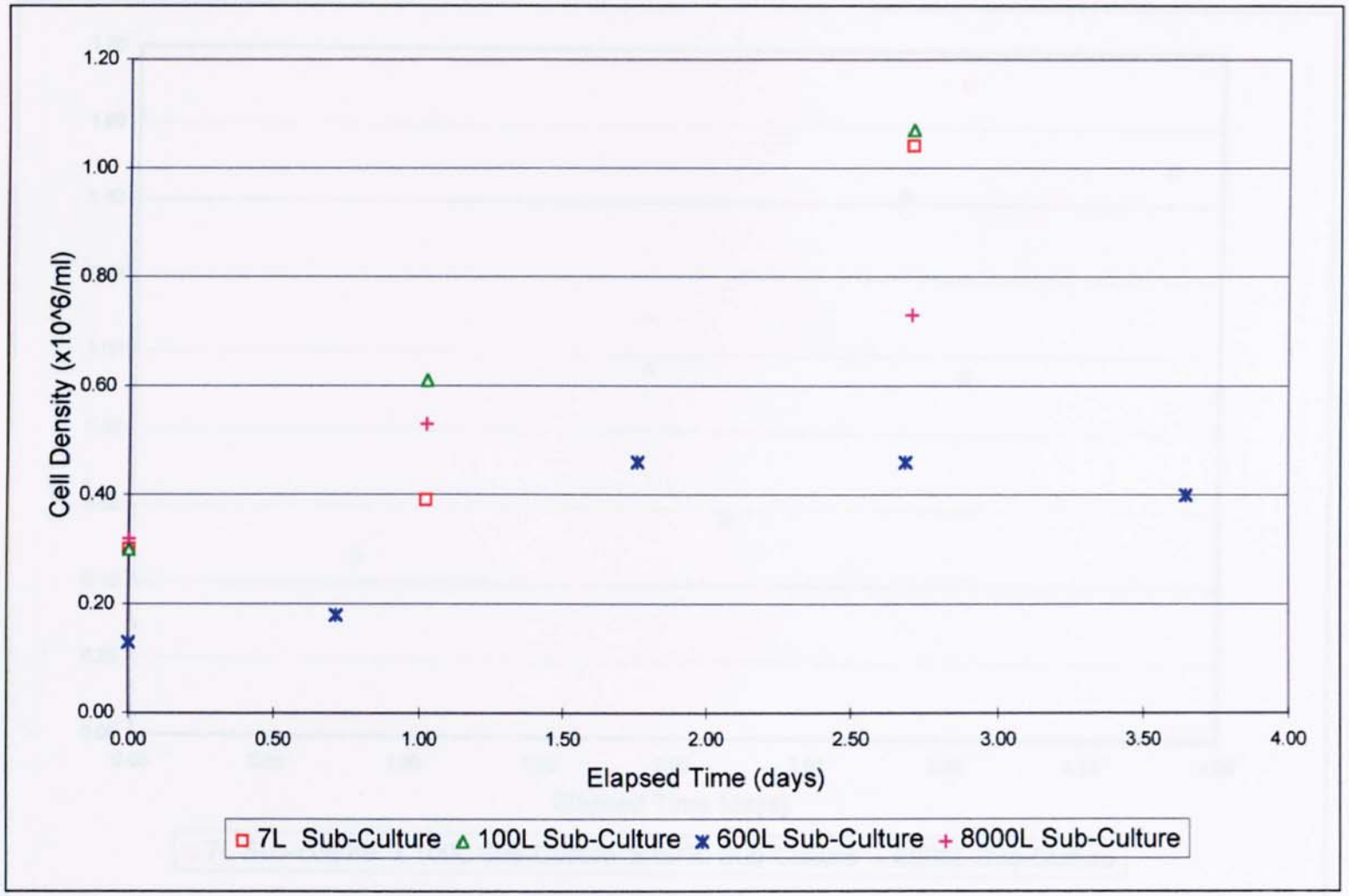

Figure 3.20 Plot of the cell densities achieved by four culture stage vessels over an operating period equivalent to the second sub-cultures of these vessels.

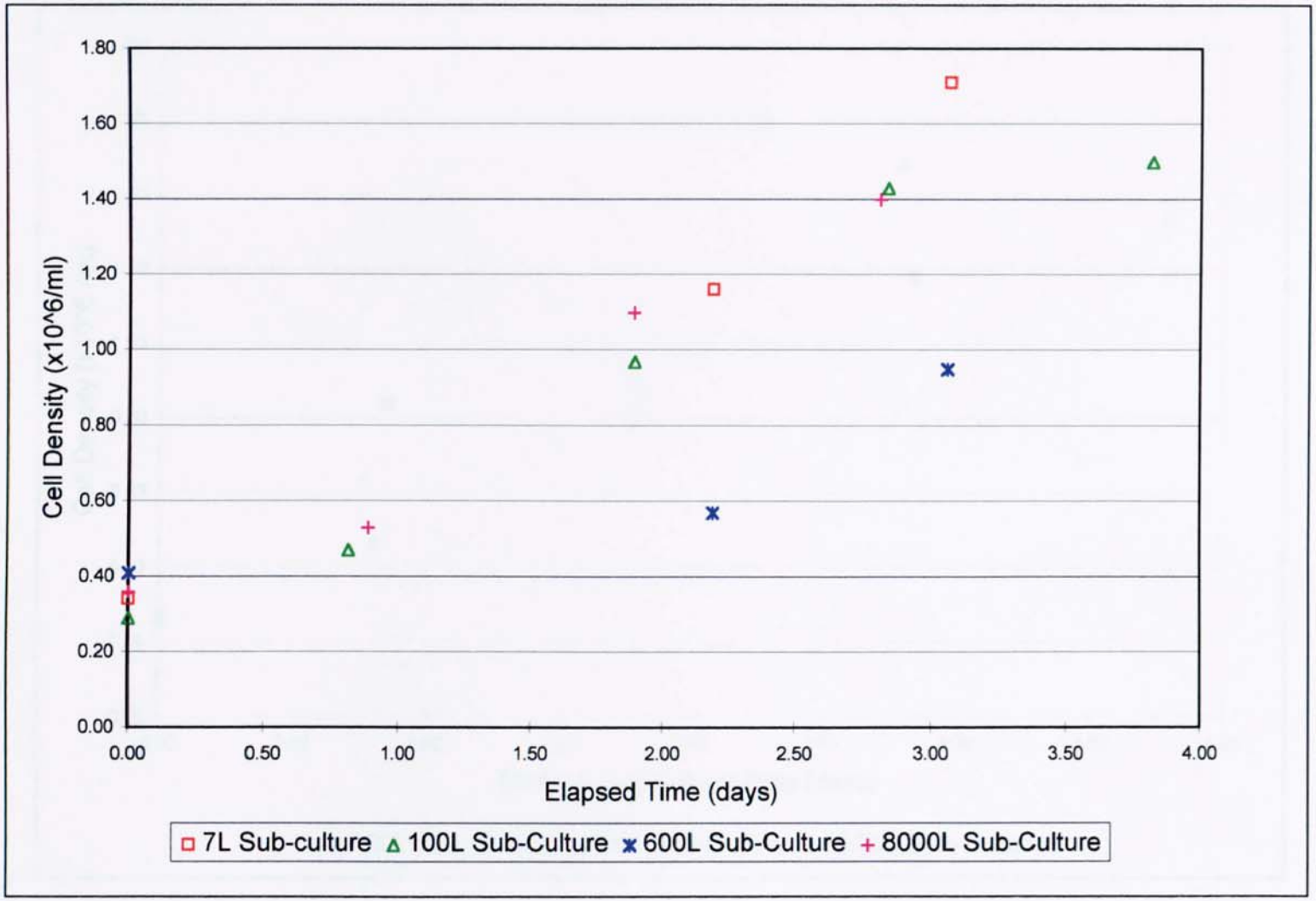

Figure 3.21 A plot of the cell densities achieved by four culture stage vessels over an operating period equivalent to the third sub-cultures of these vessels.

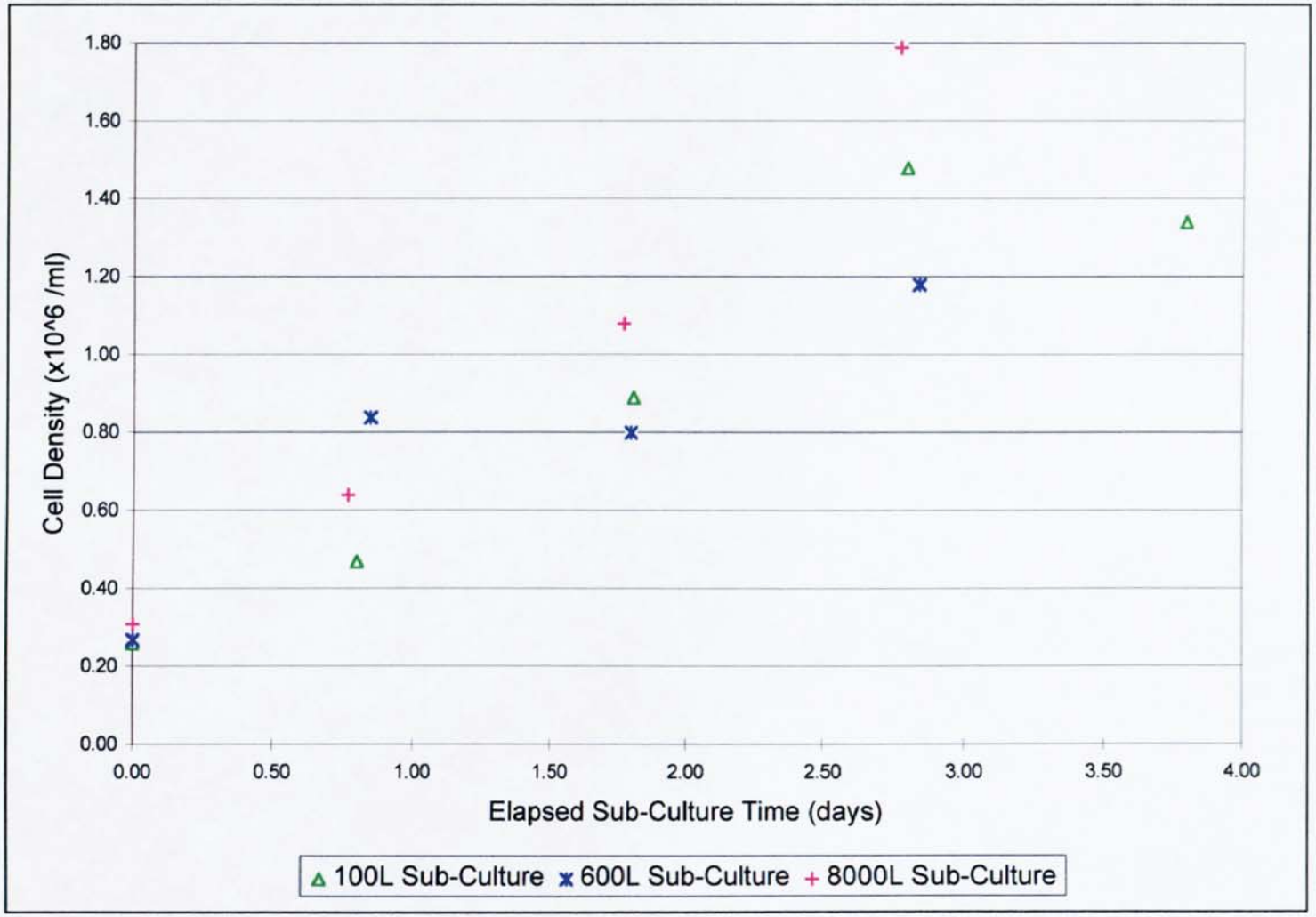

Figure 3.22 A plot of the cell densities achieved by three culture stage vessels over an operating period equivalent to the first sub-cultures of these vessels. Data obtained from the second revival of cell line.

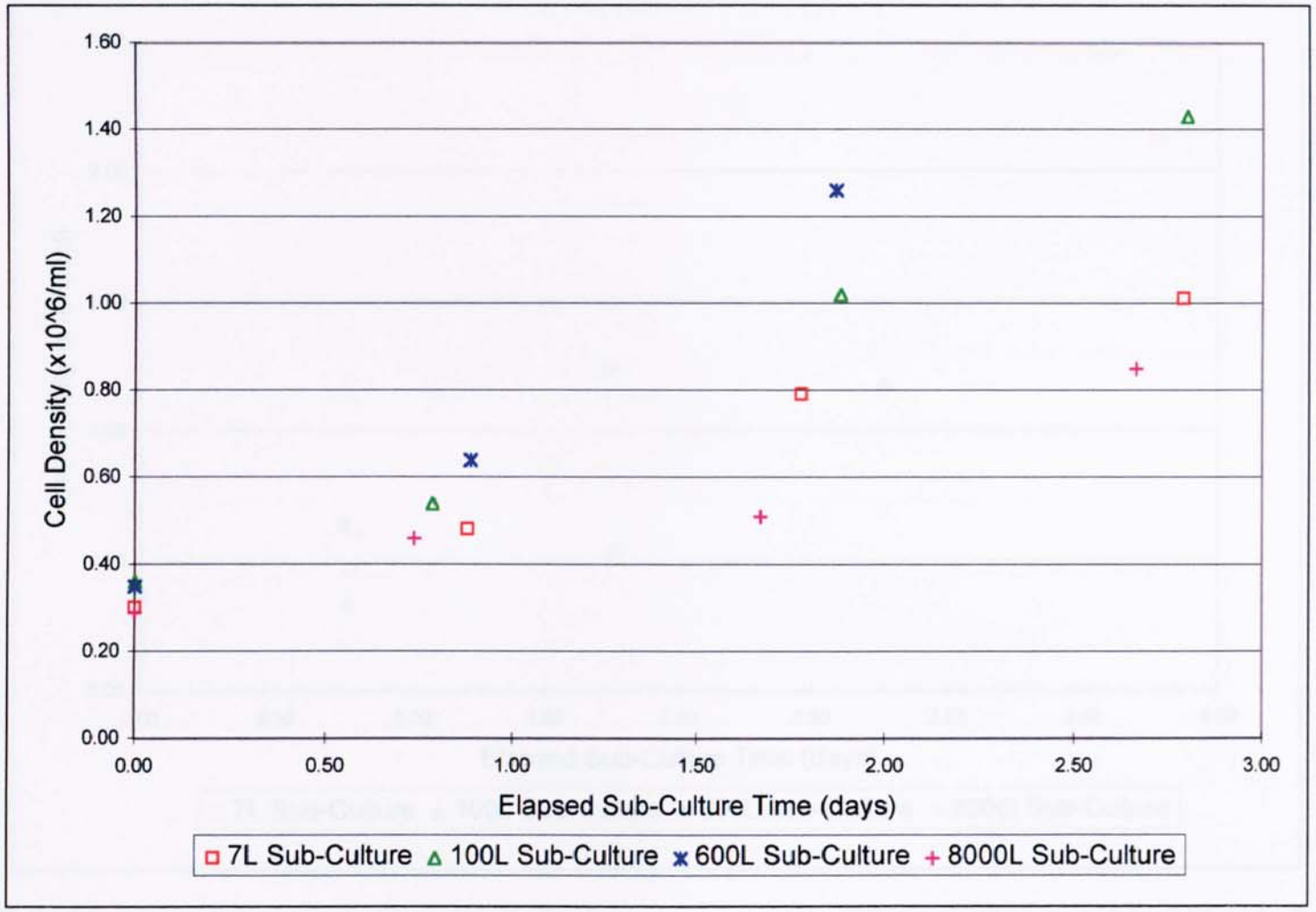

Figure 3.23 A plot of the cell densities achieved by four culture stage vessels over an operating period equivalent to the second sub-cultures of these vessels. Data obtained from the second revival of cell line.

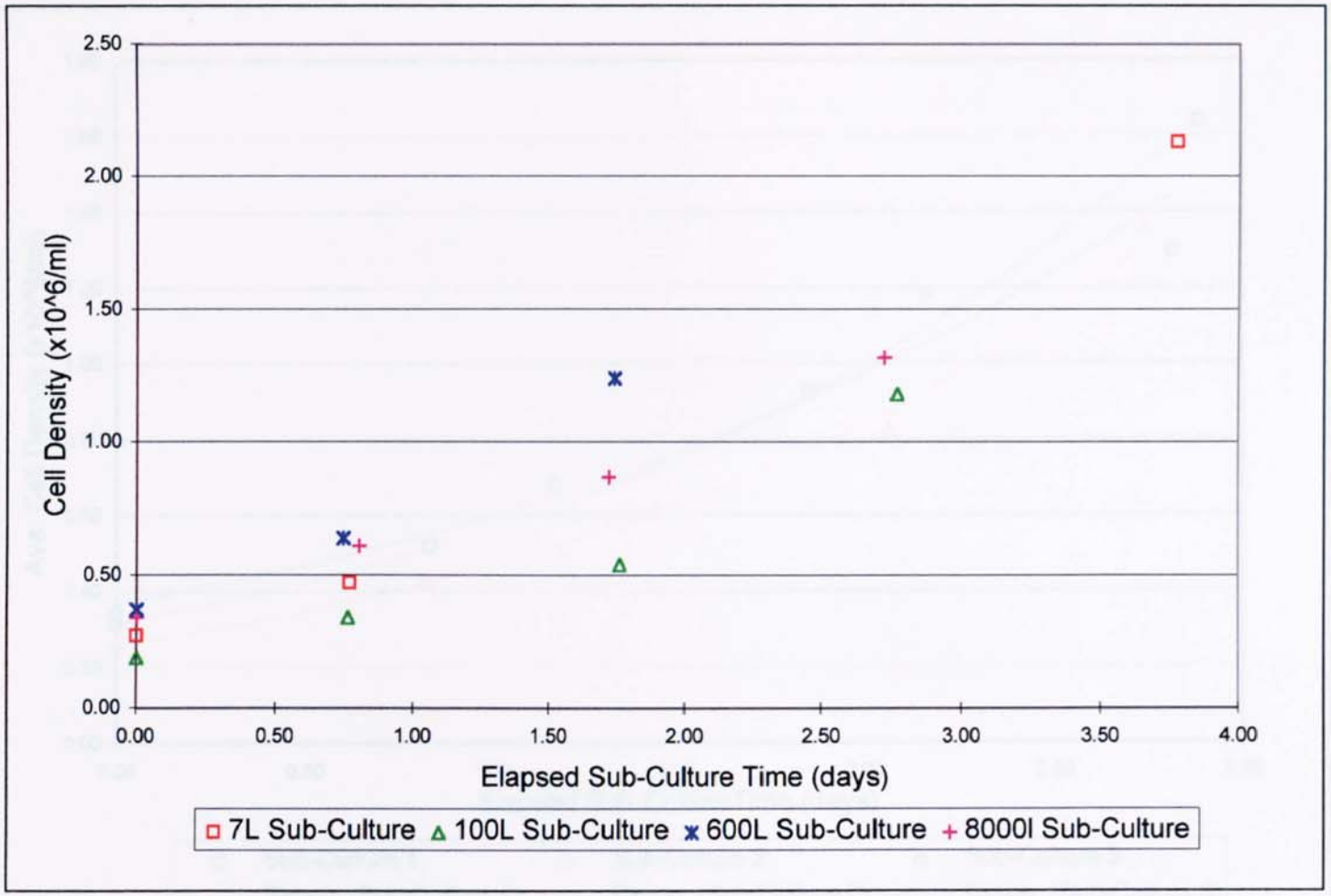

Figure 3.24 A plot of the cell densities achieved by four culture stage vessels over an operating period equivalent to the third sub-cultures of these vessels. Data obtained from the second revival of cell line.

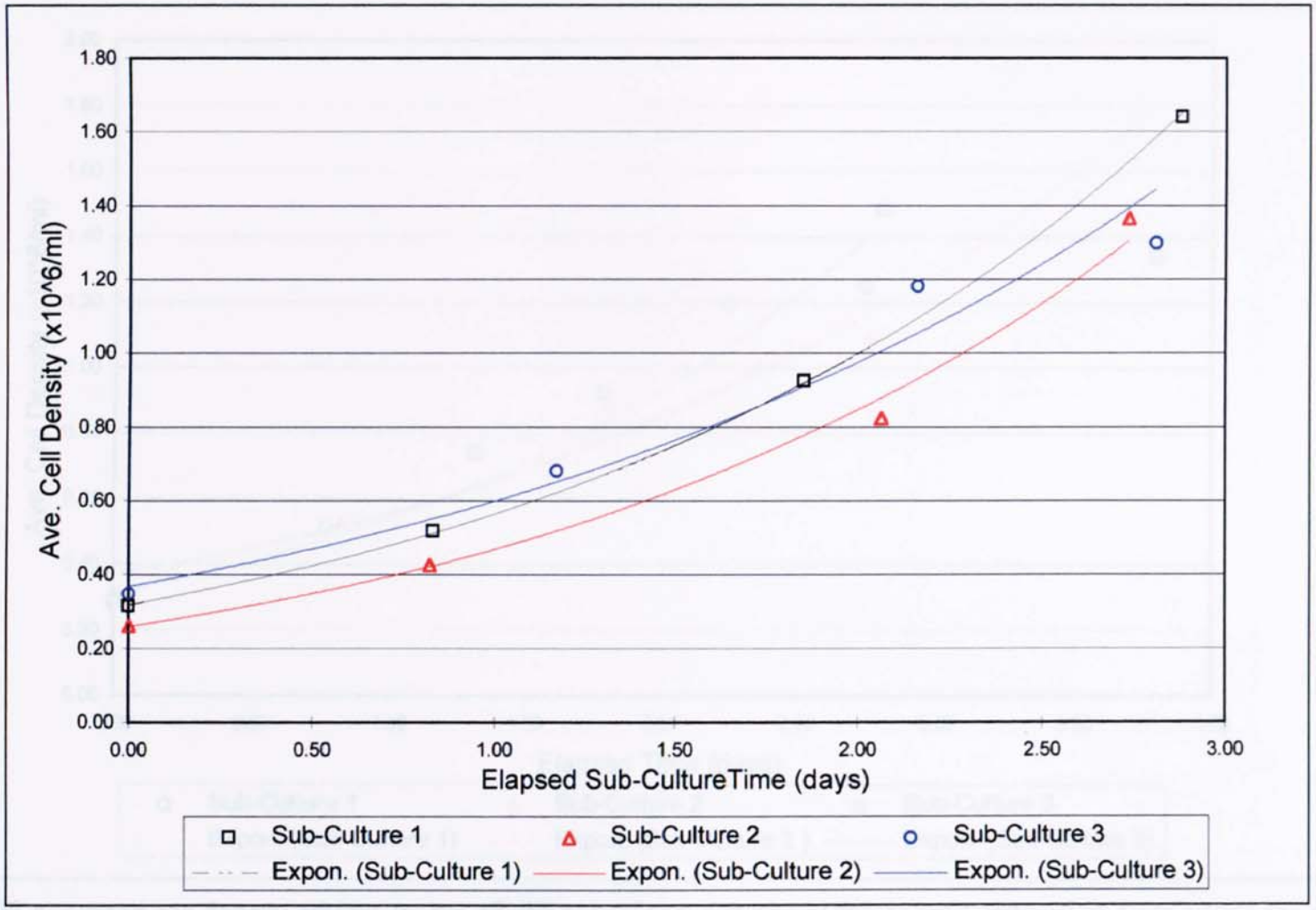

Figure 3.25 Averaged cell density profiles for the 1st, 2nd and 3rd sub-cultures of the first cell revival. Data points are overlaid with trend lines corresponding to an exponential relationship.

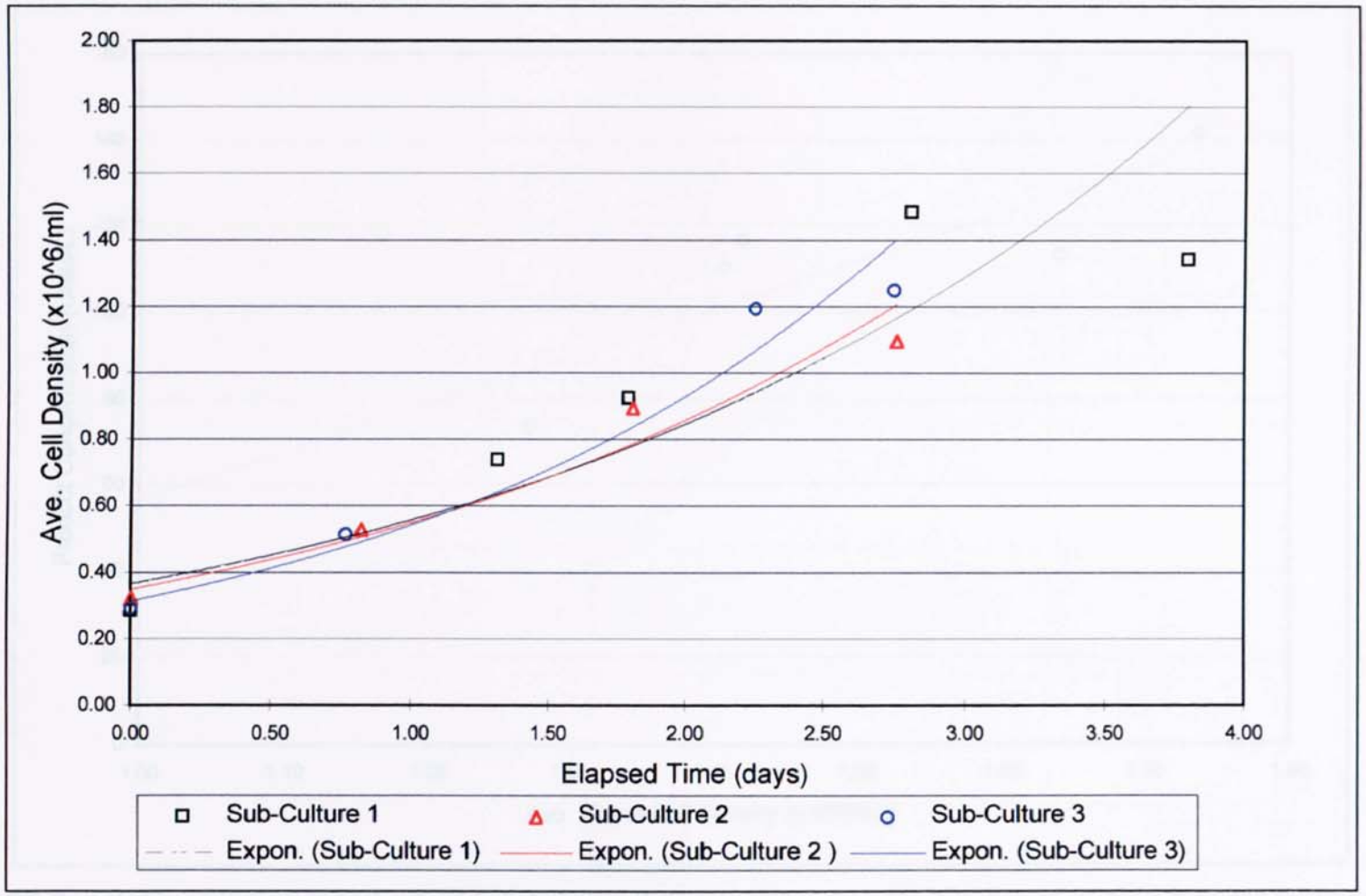

Figure 3.26 Averaged cell density profiles for the 1st, 2nd and 3rd sub-cultures of the second cell revival. Data points are overlaid with trend lines corresponding to an exponential relationship.

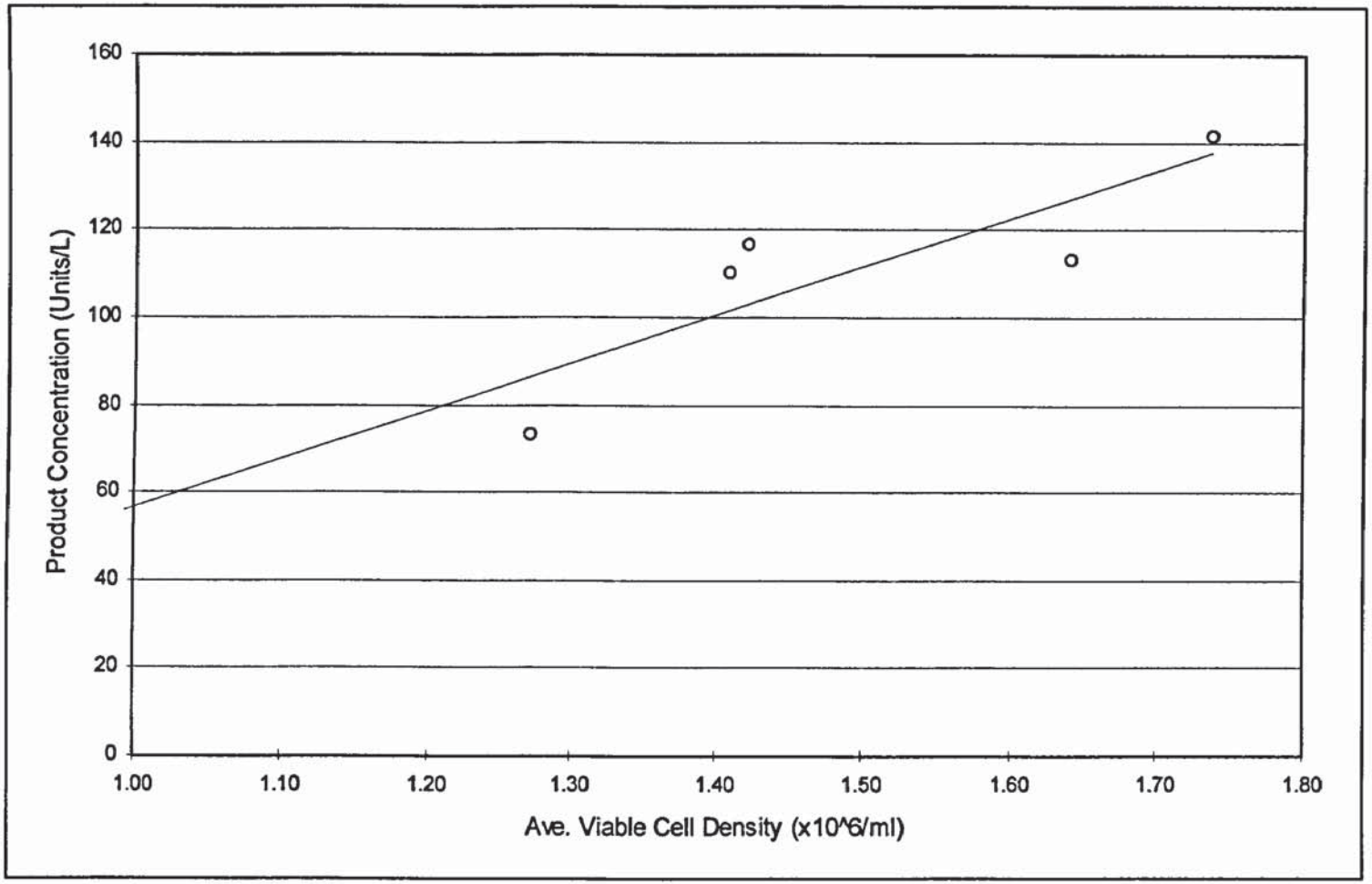

Figure 3.27 Correlation between average final cell density achieved at the end of a sub-culture and product concentration for the batch operating configuration.

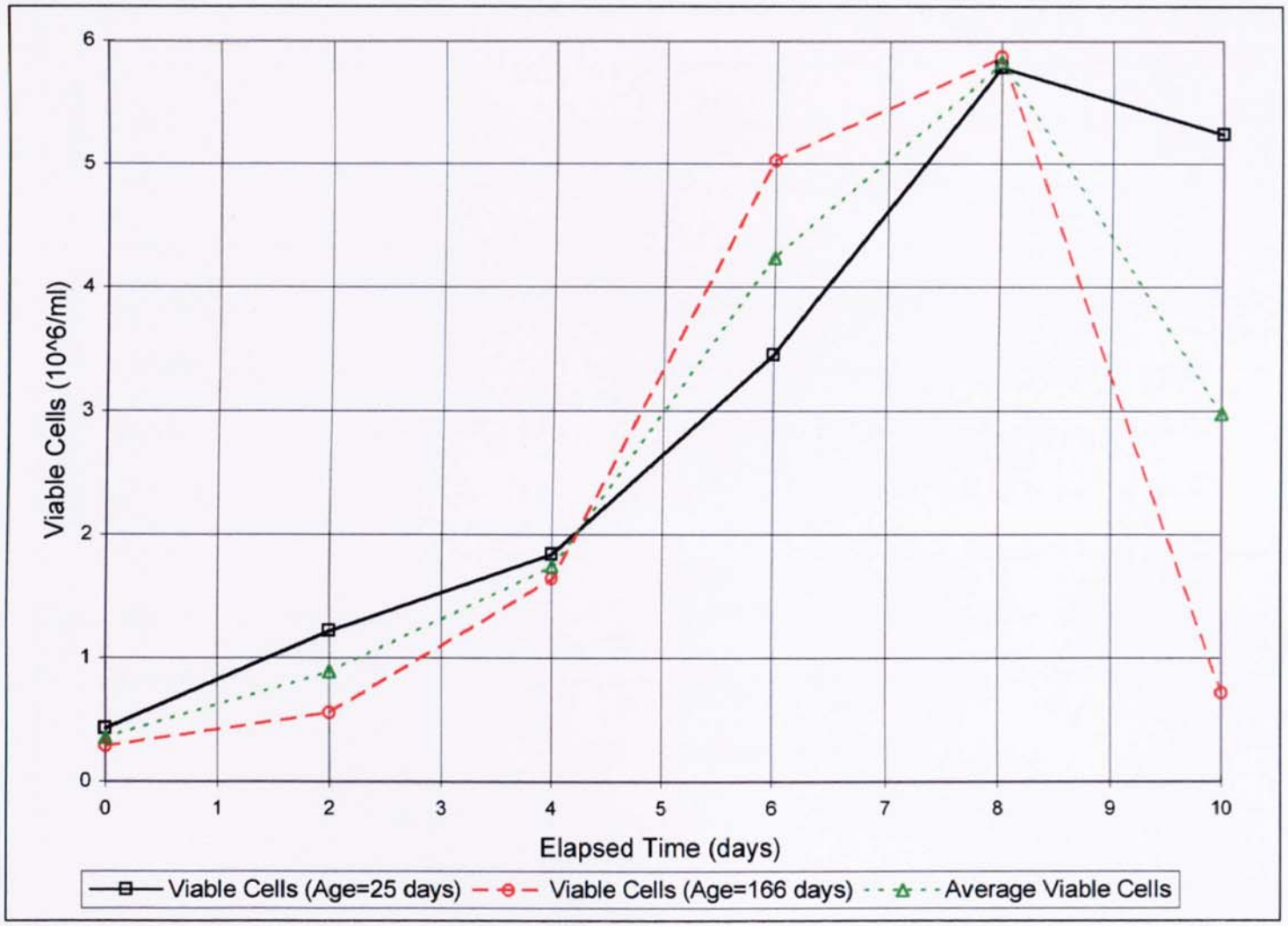

Figure 3.28 Viable cell density profile for two different culture ages for the same cell line. The average viable cell density profile of these two culture ages is also shown.

APPENDIX C (Chapter IV Figures)

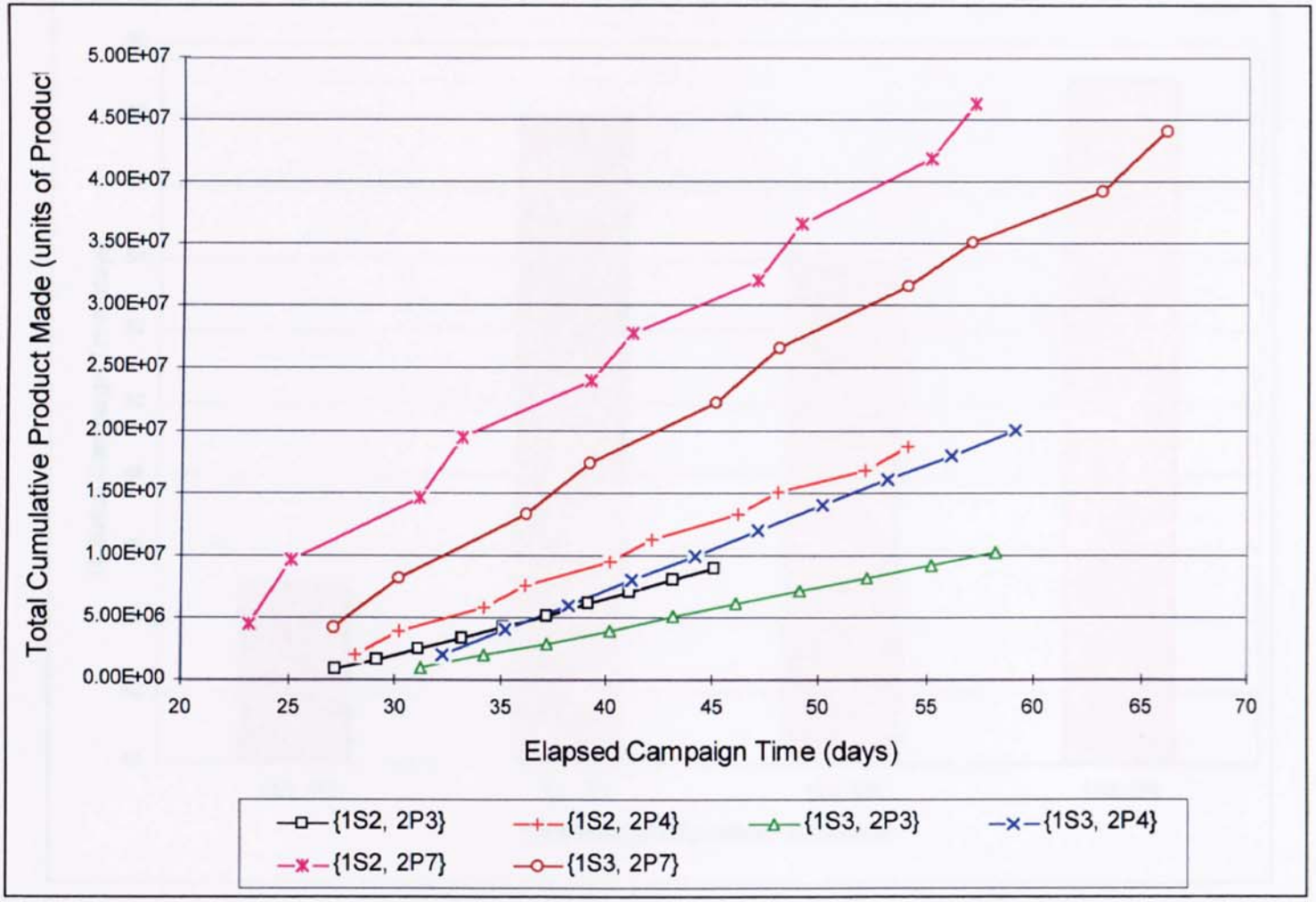

Figure 4.1 Profile showing the time taken to produce a given amount of product by each of the batch and fed-batch operating schedules studied. This performance parameter is known commonly as the product makespan.

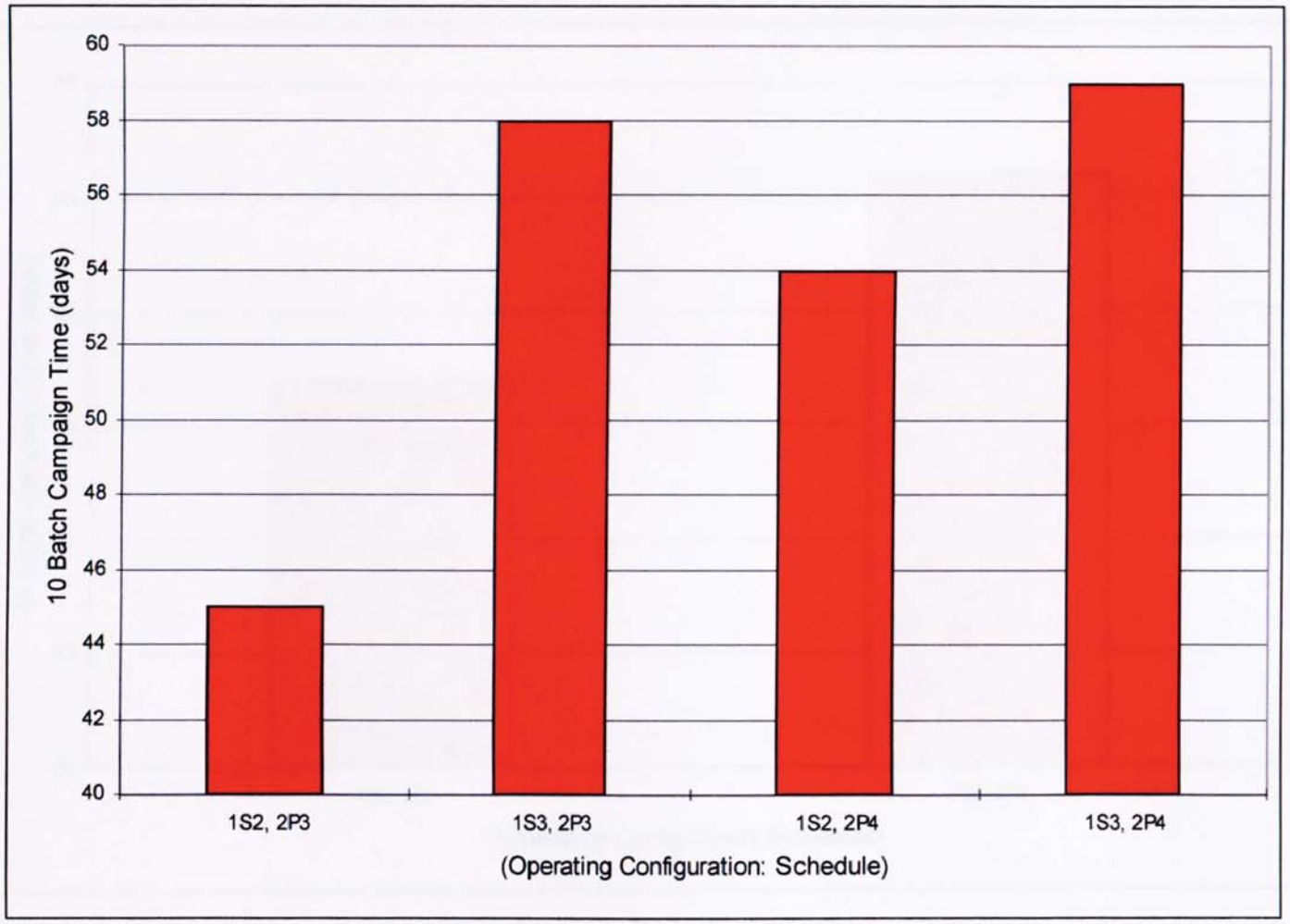

Figure 4.2 Comparison profile showing the simulated average total time taken to produce 10 complete product batches by each of the 4 Batch operating configuration schedules studied.

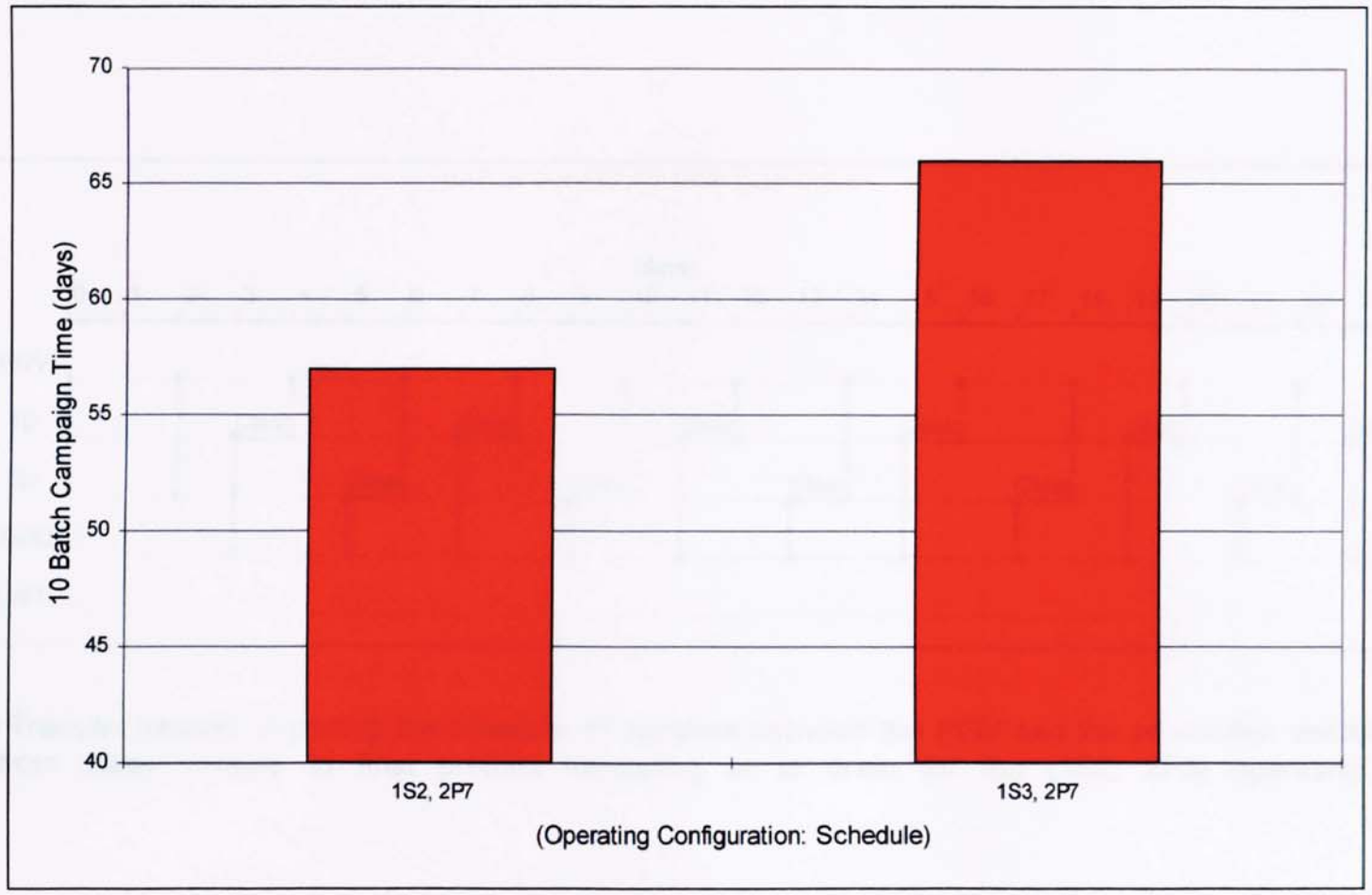

Figure 4.3 Comparison profile showing the simulated average total time taken to produce 10 complete product batches by the 2 Fed-batch operating configuration schedules studied.

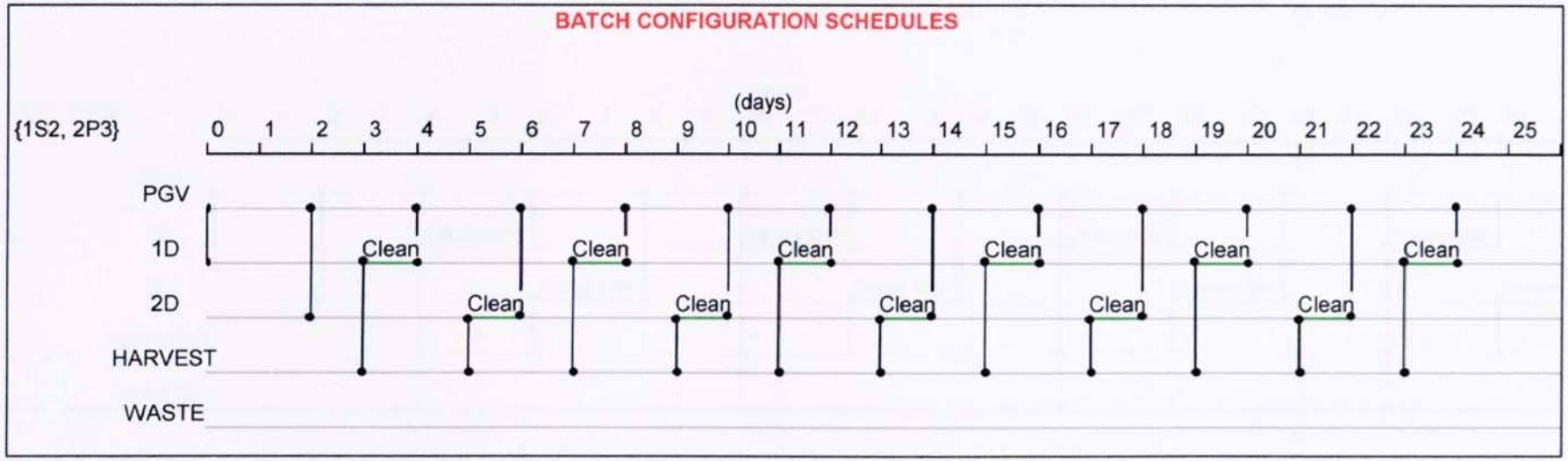

Figure 4.4 Transfer network depicting the schedule of transfers between the PGV and the production vessels (1D and 2D) and from these vessels to final product harvesting or to drain for the {1S2, 2P3} operating schedule.

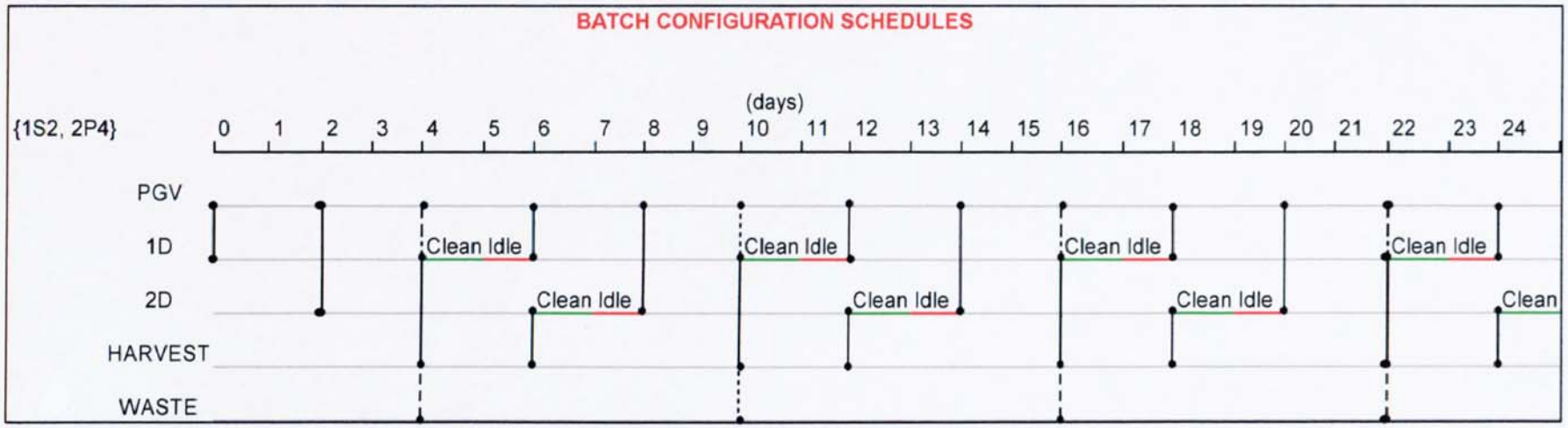

Figure 4.5 Transfer network depicting the schedule of transfers between the PGV and the production vessels (1D and 2D) and from these vessels to final product harvesting or to drain for the {1S2, 2P4} operating schedule.

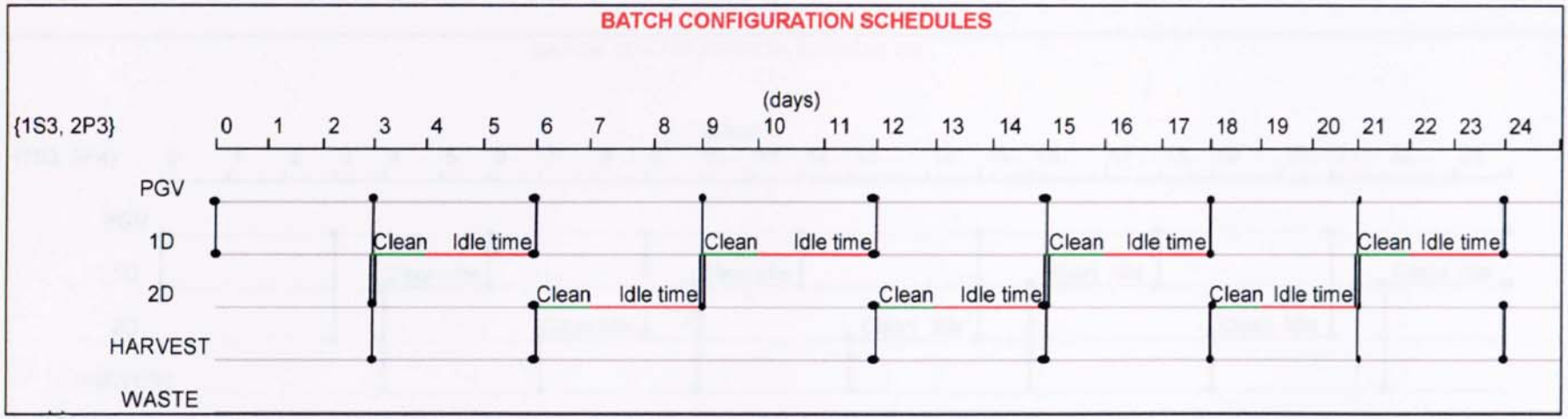

Figure 4.6 Transfer network depicting the schedule of transfers between the PGV and the production vessels (1D and 2D) and from these vessels to final product harvesting or to drain for the {1S3, 2P3} operating schedule.

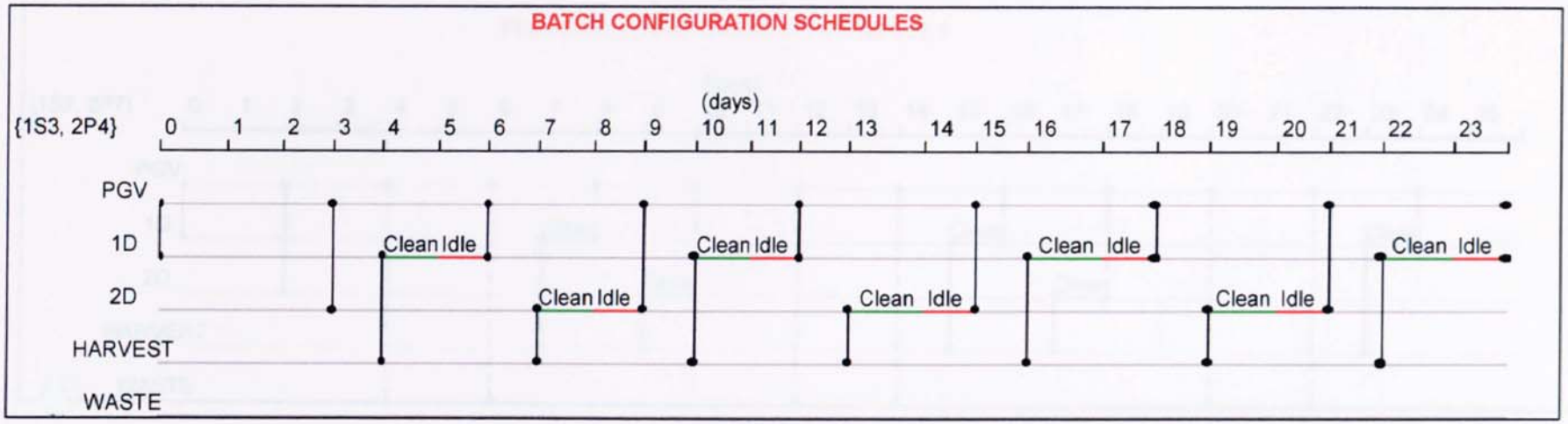

Figure 4.7 Transfer network depicting the schedule of transfers between the PGV and the production vessels (1D and 2D) and from these vessels to final product harvesting or to drain for the {1S3, 2P4} operating schedule.

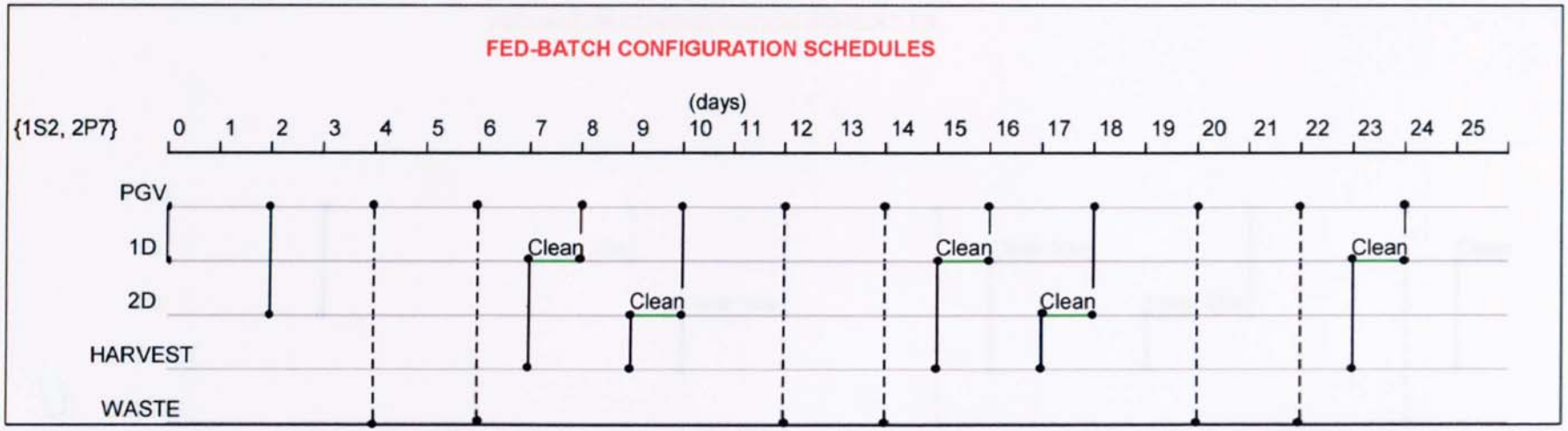

Figure 4.8 Transfer network depicting the schedule of transfers between the PGV and the production vessels (1D and 2D) and from these vessels to final product harvesting or to drain for the {1S2, 2P7} operating schedule.

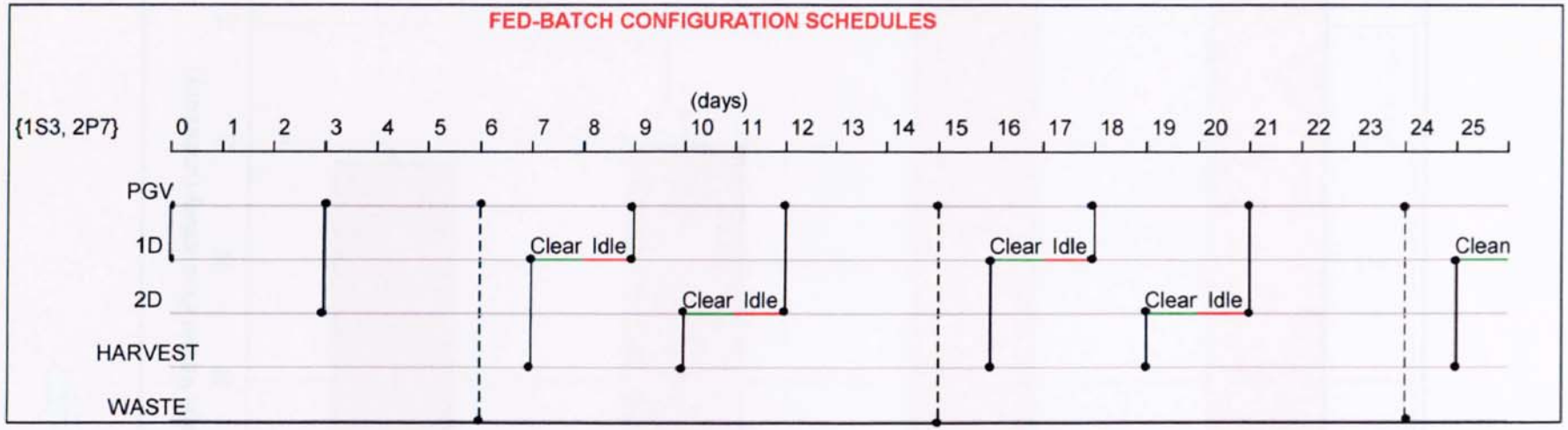

Figure 4.9 Transfer network depicting the schedule of transfers between the PGV and the production vessels (1D and 2D) and from these vessels to final product harvesting or to drain for the {1S3, 2P7} operating schedule.

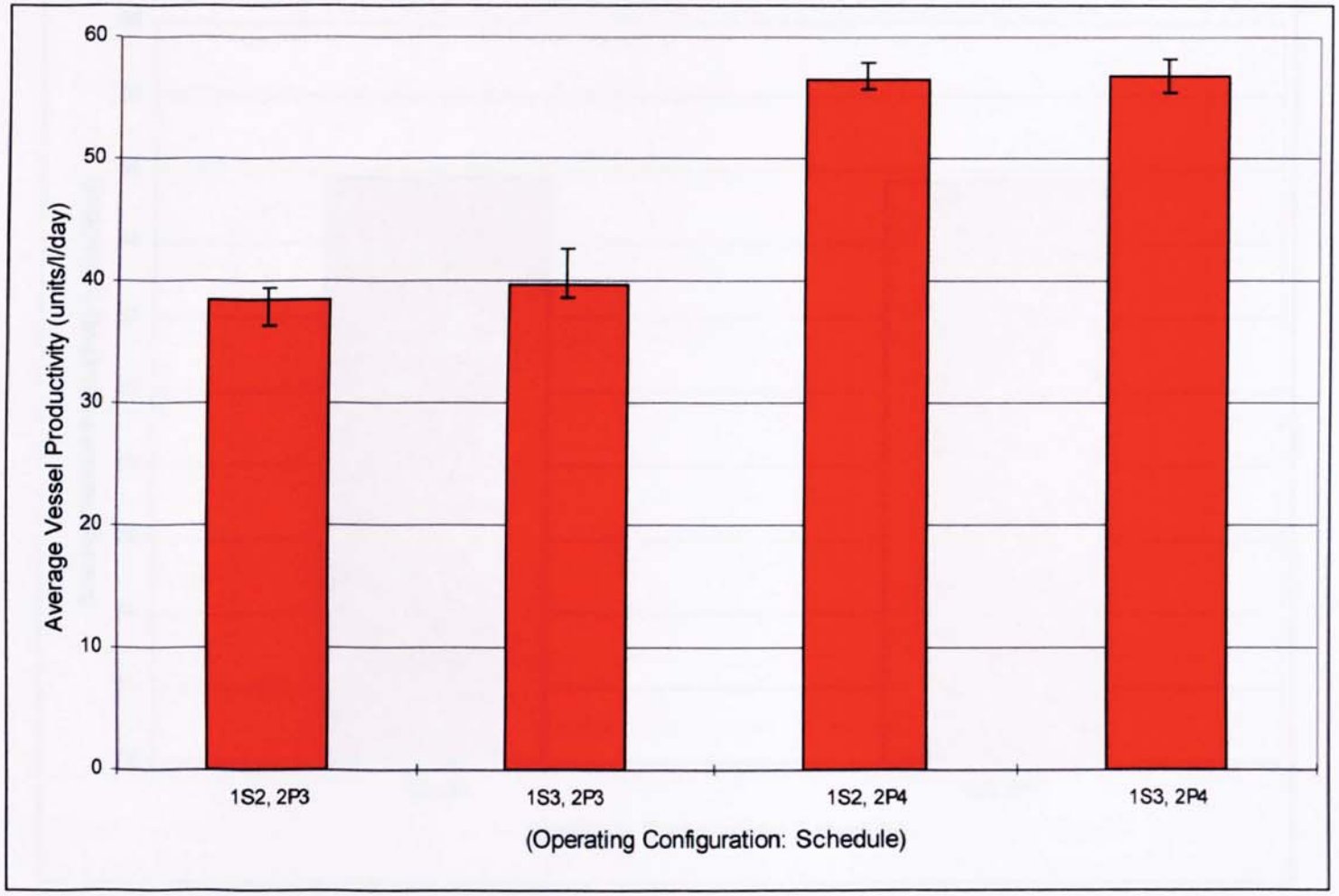

Figure 4.10 Profile comparing the average production vessel productivity for each of the 4 simulated Batch operating configuration schedules. The average values being based upon the completion of 10 product batches for all the operating schedules studied.

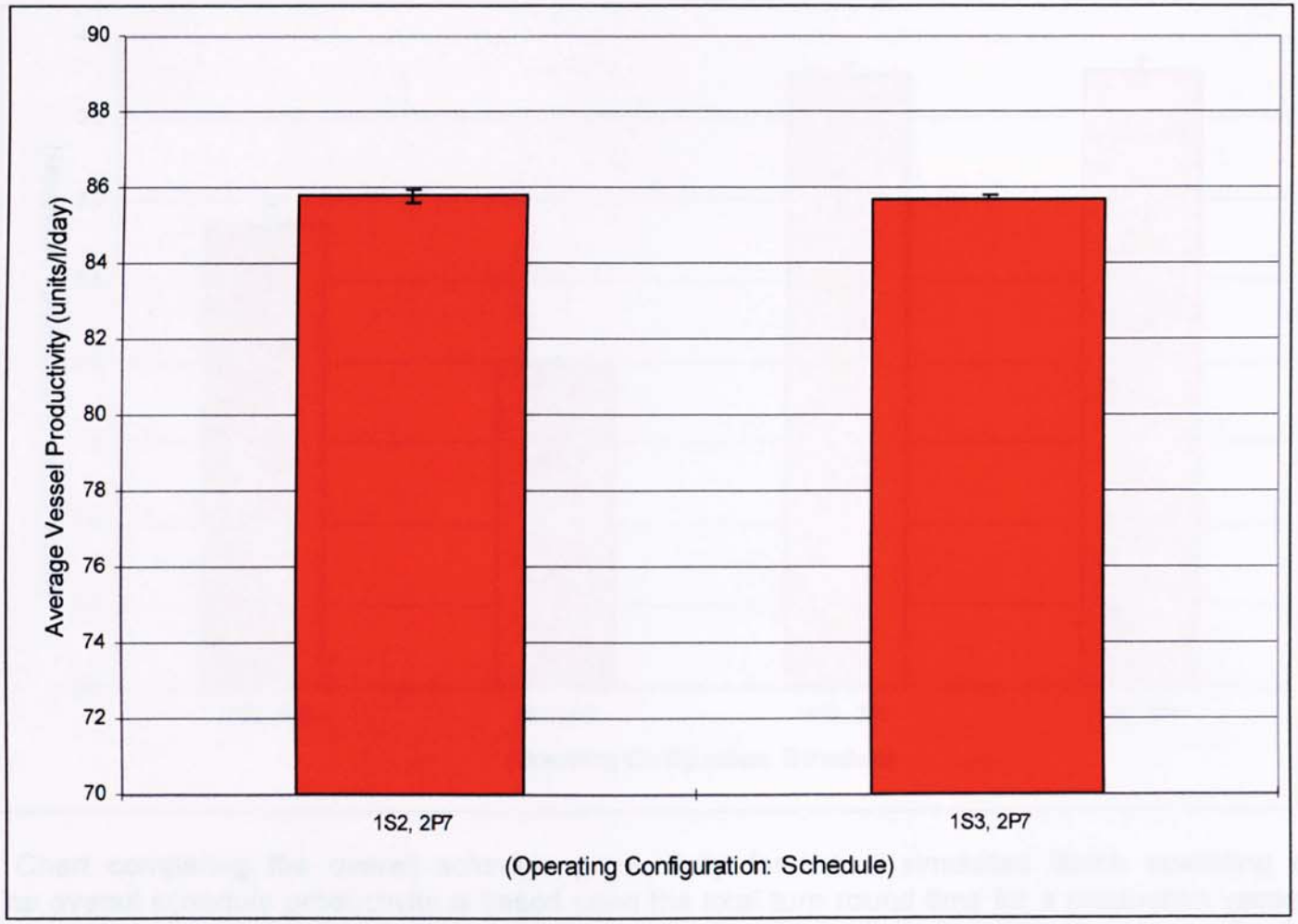

Figure 4.11 Profile comparing the average production vessel productivity for the 2 simulated Fed-batch operating configuration schedules. The average values being based upon the completion of 10 product batches for both of the operating schedules studied.

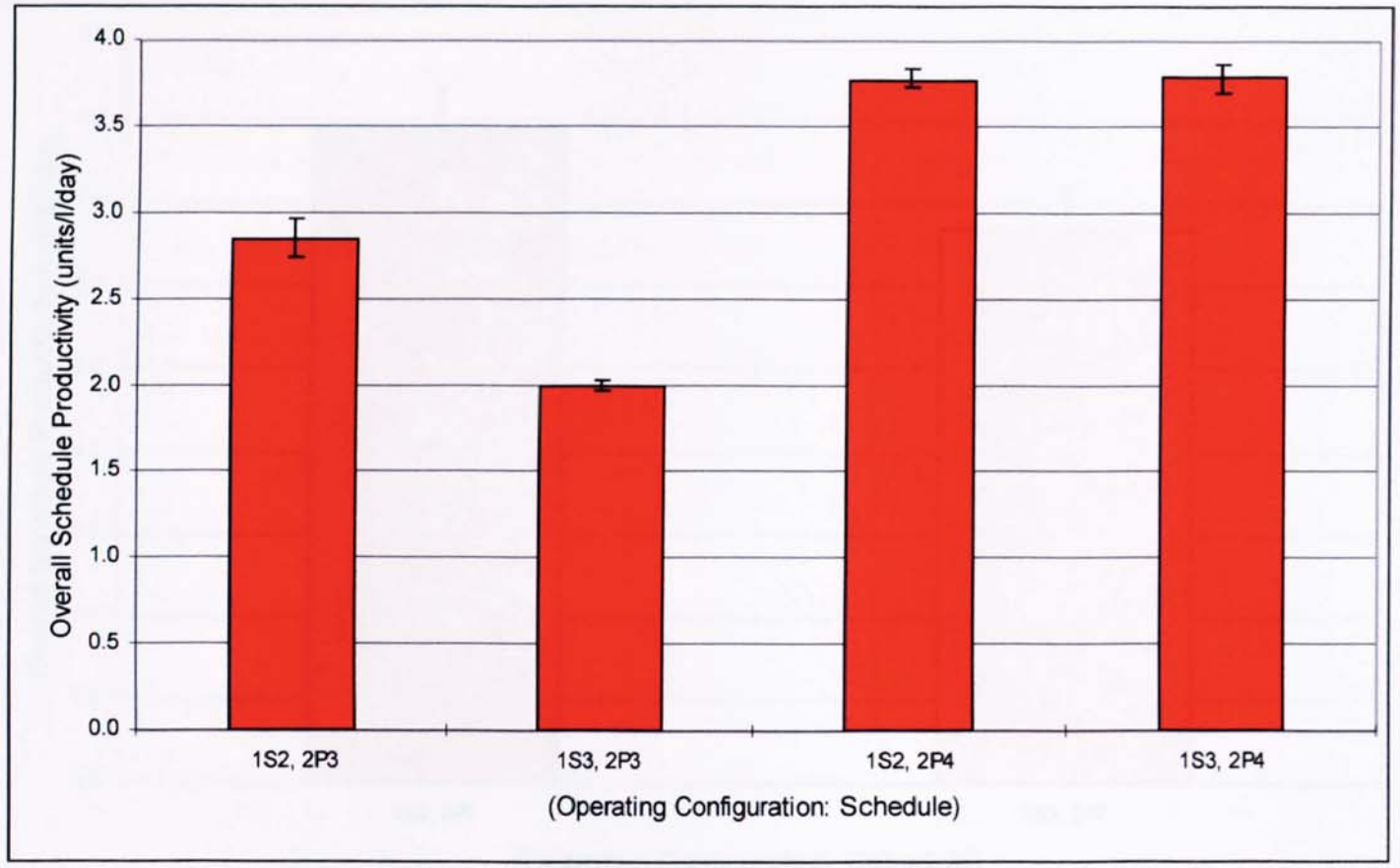

Figure 4.12 Chart comparing the overall schedule productivity for the 4 simulated Batch operating configuration schedules. The overall schedule productivity is based upon the total turn round time for a production vessel. Therefore, the overall schedule productivity takes into account the production vessel cleaning time and idle time between successive product batches.

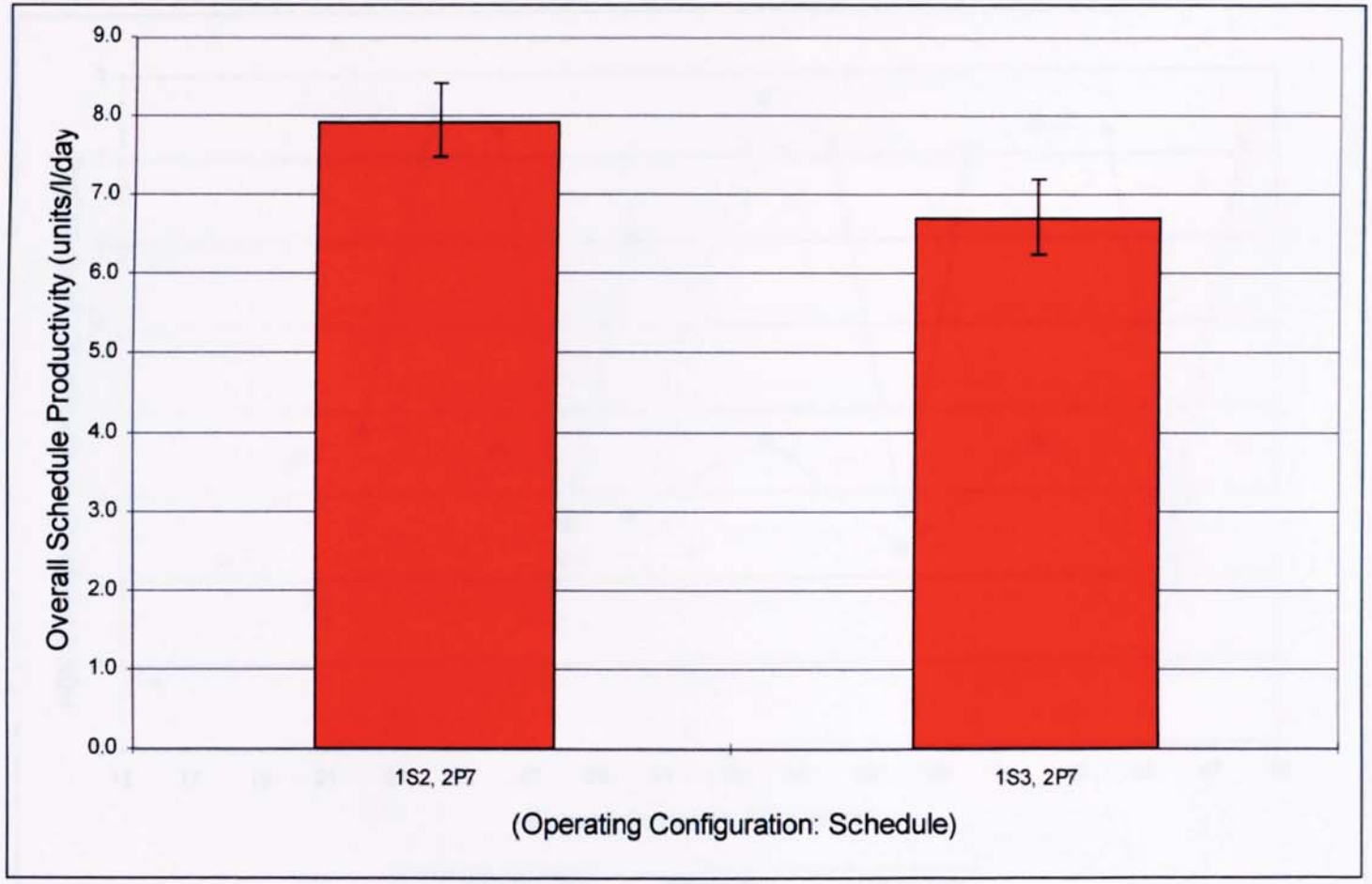

Figure 4.13 Comparison of the overall schedule productivity for both of the Fed-batch operating configuration schedules. The overall schedule productivity is based upon the total turn round time for a production vessel. Therefore, the overall schedule productivity takes into account the production vessel cleaning time and idle time between successive product batches.

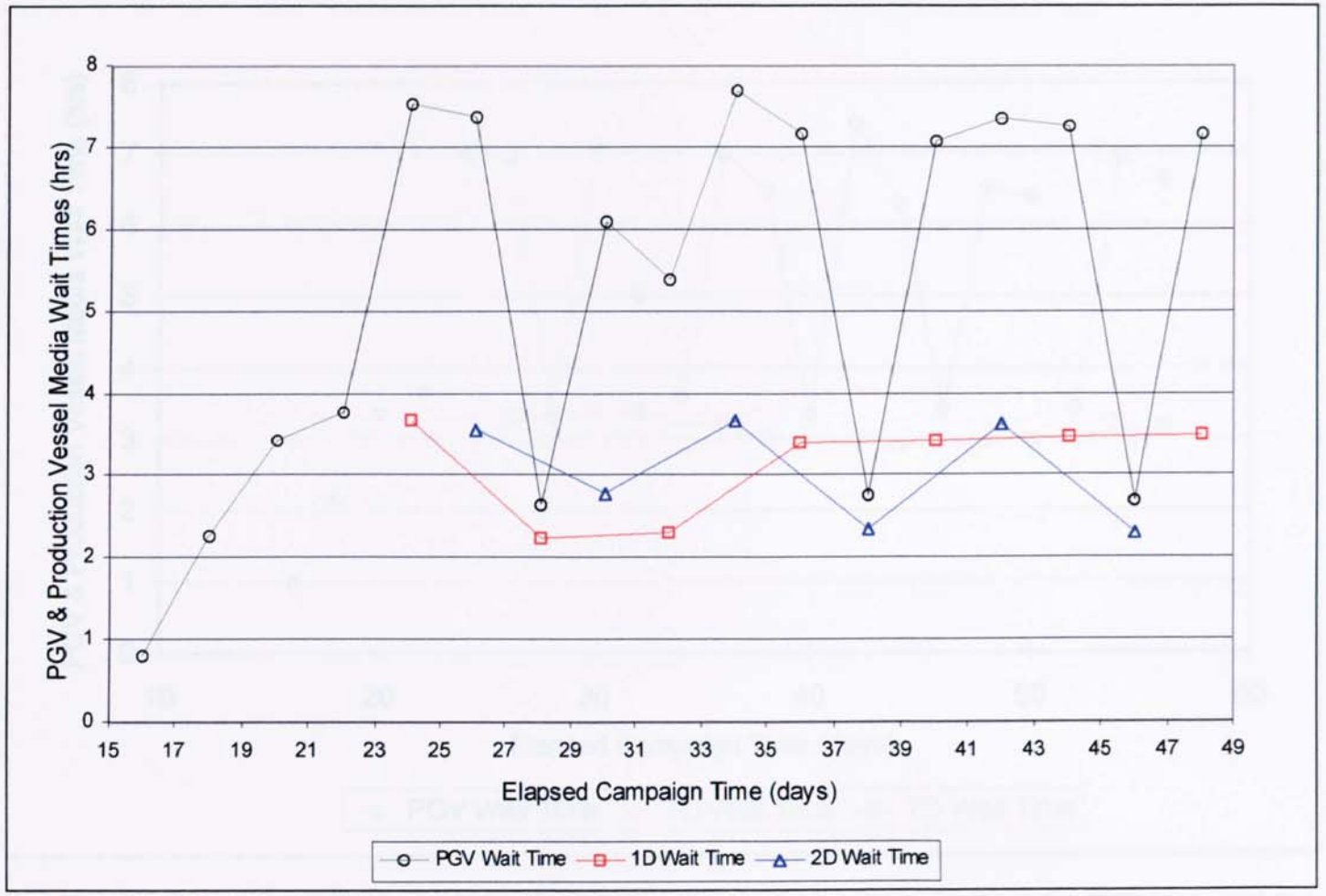

Figure 4.14 Trend chart showing difference in the media waiting times experienced by the PGV (O) and the production vessels (Δ and □) over an entire production campaign. This profile represents the characteristic behaviour for the {1S2, 2P3} schedule, operating under a Batch process configuration.

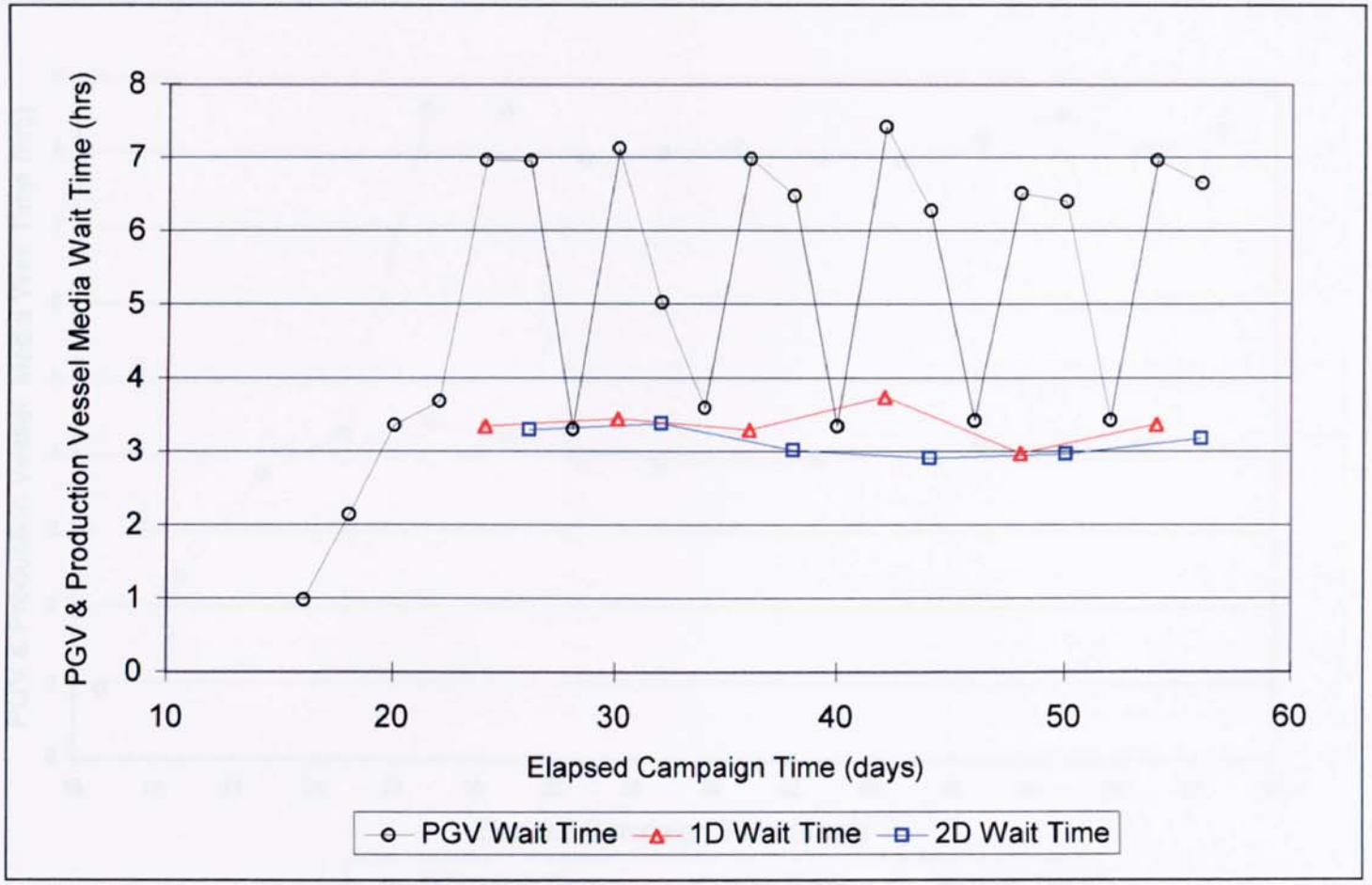

Figure 4.15 Trend chart showing difference in the media waiting times experienced by the PGV (O) and the production vessels (Δ and □) over an entire production campaign. This profile represents the characteristic behaviour for the {1S2, 2P4} schedule, operating under a Batch process configuration.

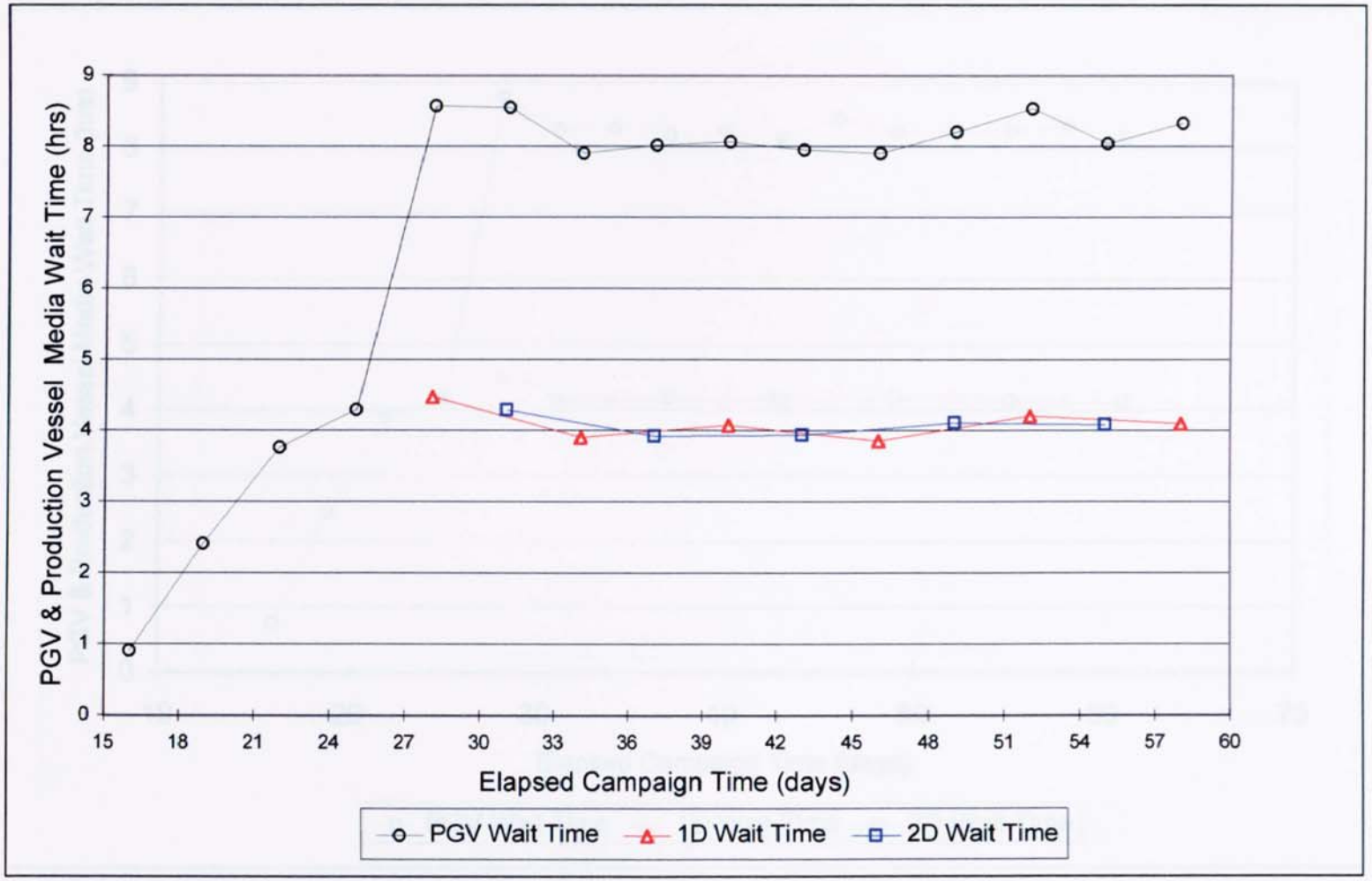

Figure 4.16 Trend chart showing difference in the media waiting times experienced by the PGV (O) and the production vessels (Δ and □) over an entire production campaign. This profile represents the characteristic behaviour for the {1S3, 2P3} schedule, operating under a Batch process configuration.

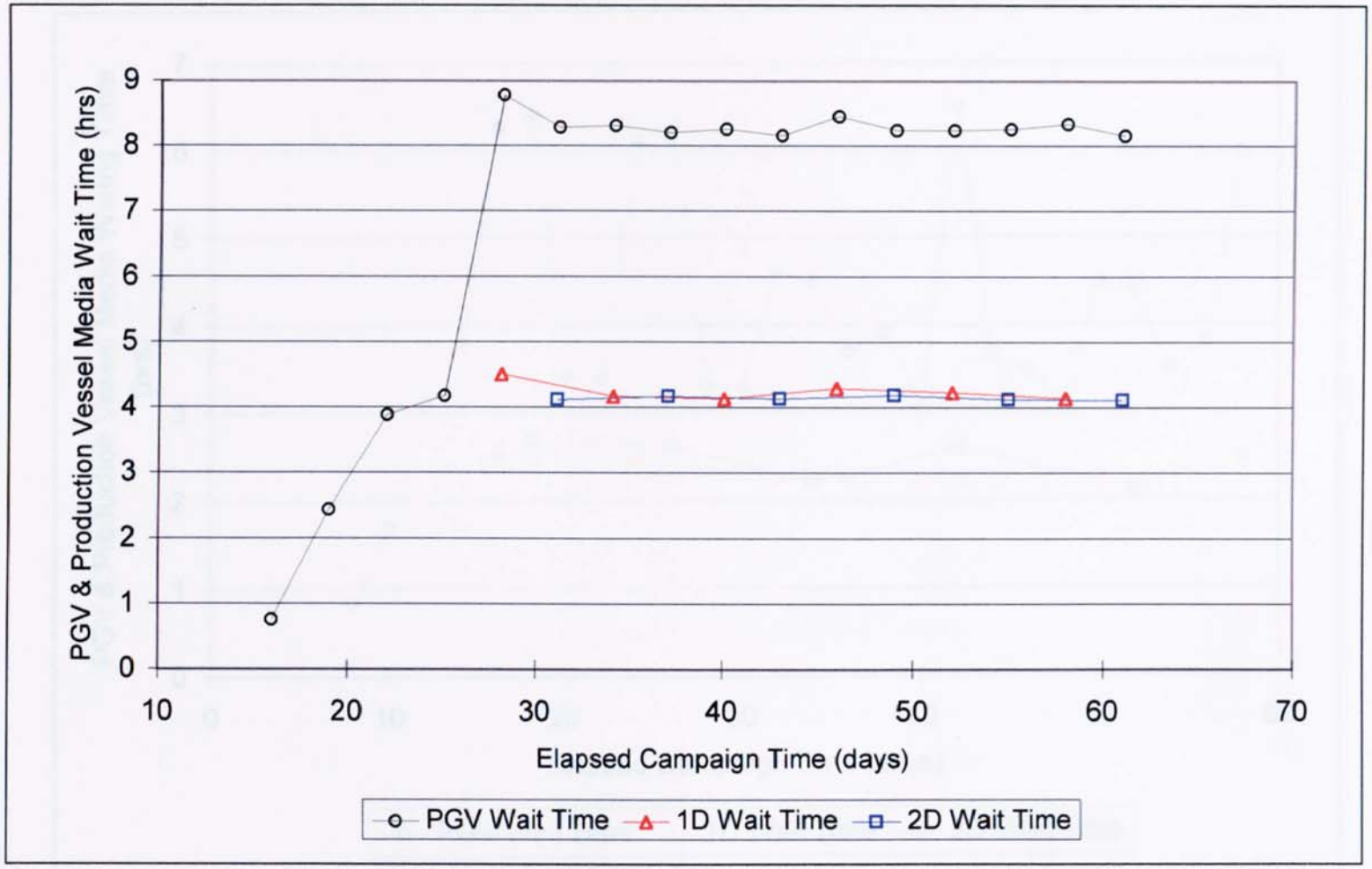

Figure 4.17 Trend chart showing difference in the media waiting times experienced by the PGV (O) and the production vessels (Δ and □) over an entire production campaign. This profile represents the characteristic behaviour for the {1S2, 2P4} schedule, operating under a Batch process configuration.

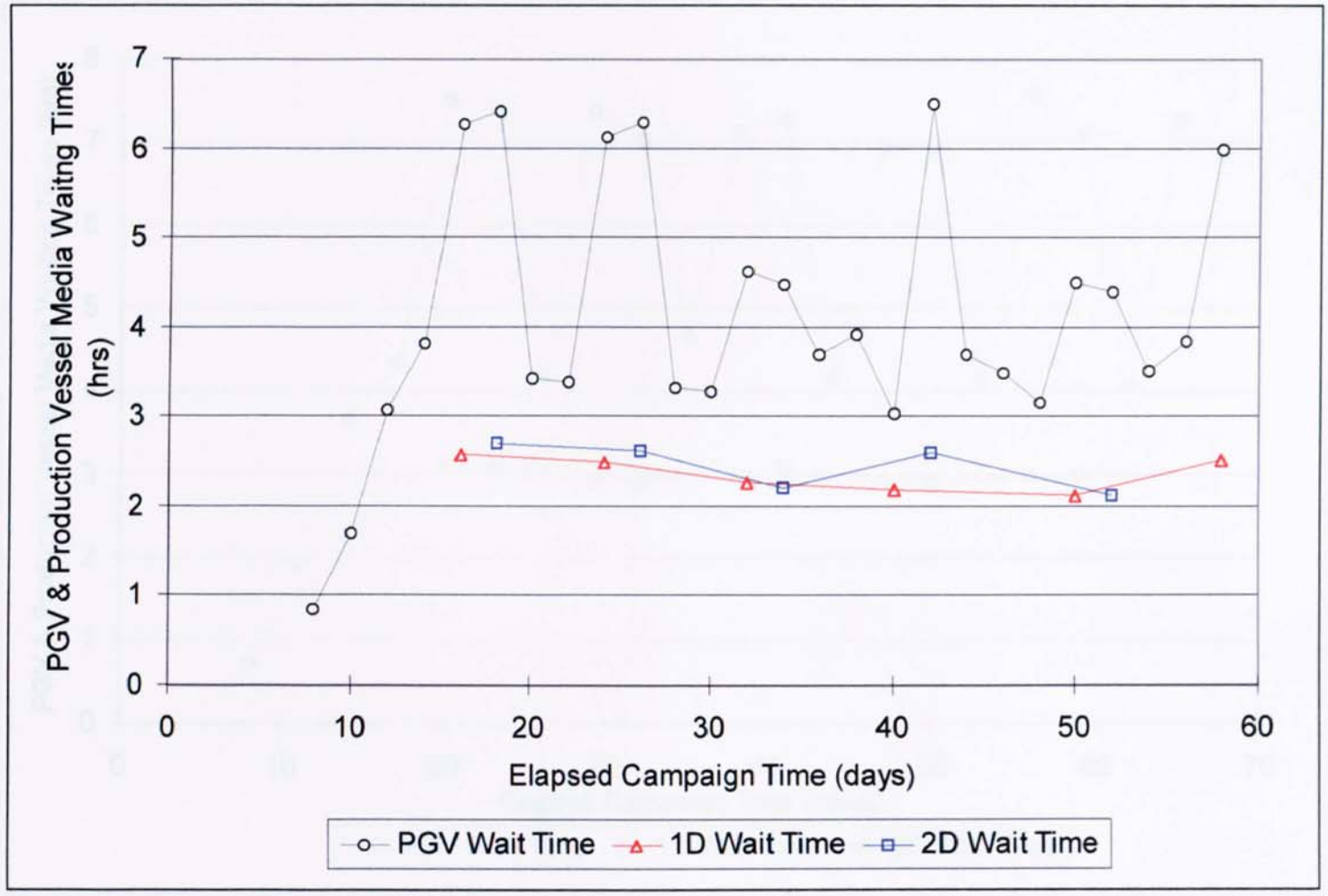

Figure 4.18 Trend chart showing difference in the media waiting times experienced by the PGV (O) and the production vessels (Δ and □) over an entire production campaign. This profile represents the characteristic behaviour for the {1S2, 2P7} schedule, operating under a Fed-batch process configuration.

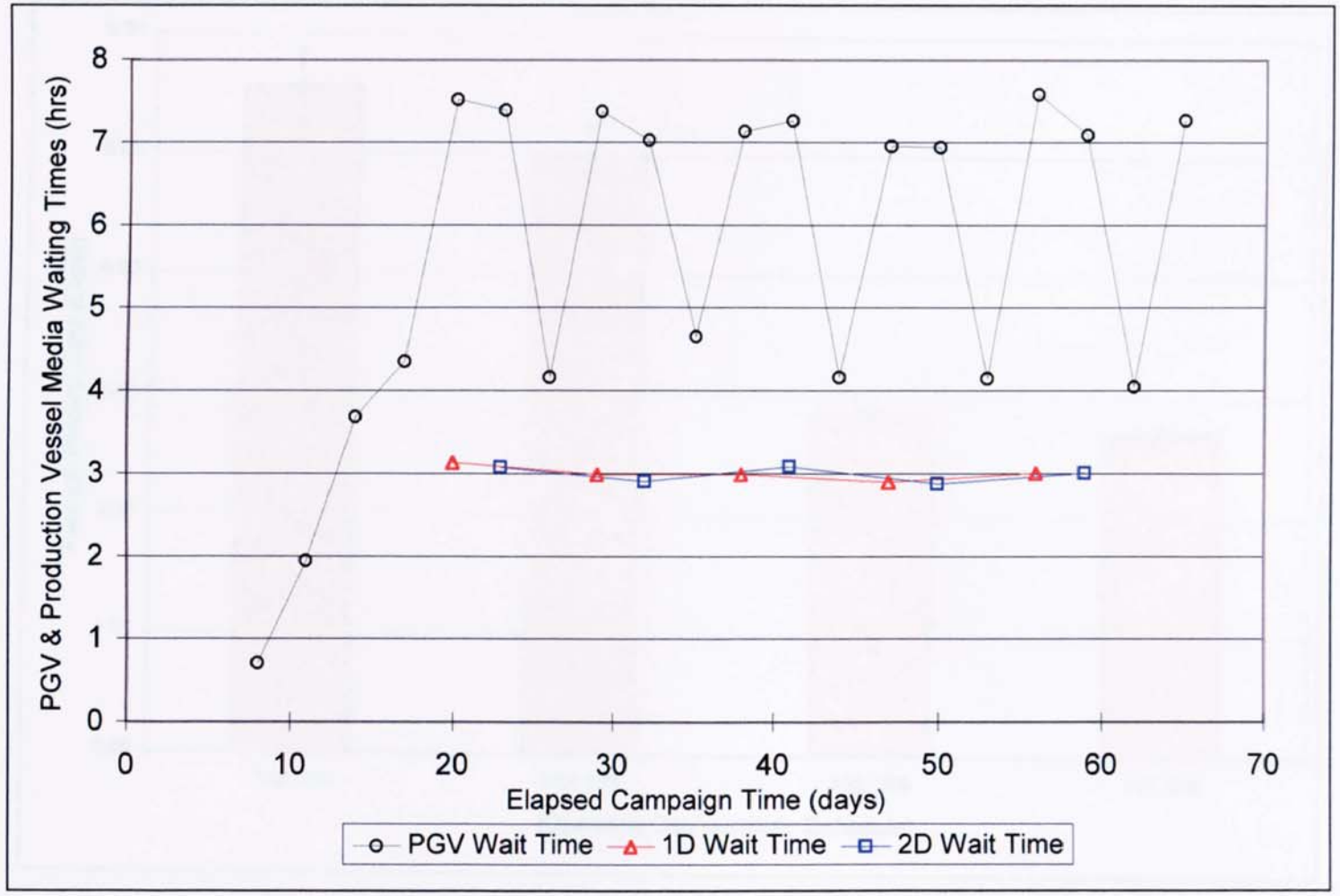

Figure 4.19 Trend chart showing difference in the media waiting times experienced by the PGV (O) and the production vessels (Δ and □) over an entire production campaign. This profile represents the characteristic behaviour for the {1S3, 2P7} schedule, operating under a Fed-batch process configuration.

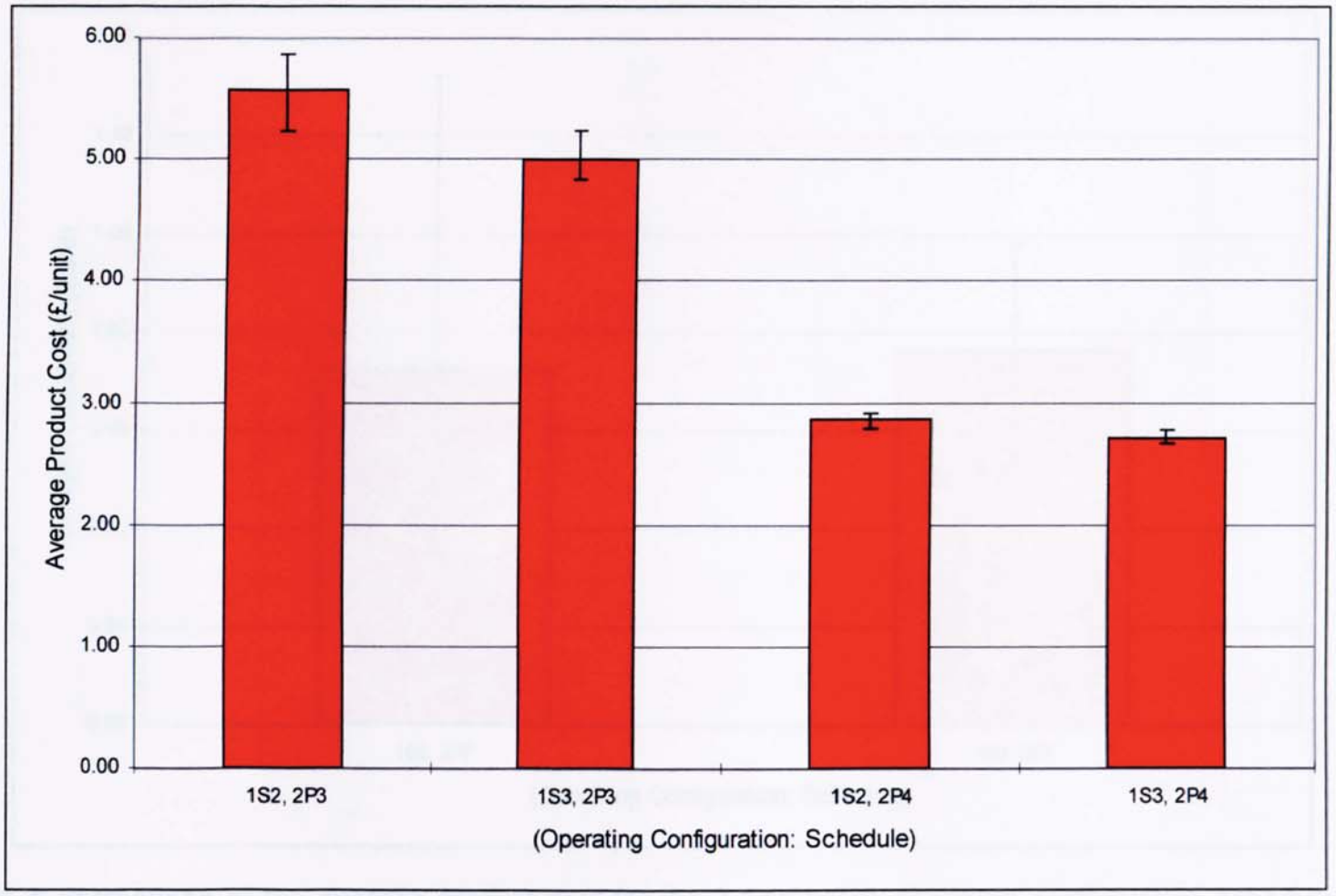

Figure 4.20 Summary profile comparing the average product cost for the 4 Batch operating configuration schedules. Average cost based upon the production of 10 complete batches of product. The size of each batch produced for a given operating schedule will not vary significantly, so the profile can then be assumed to represent the average product cost per batch of product.

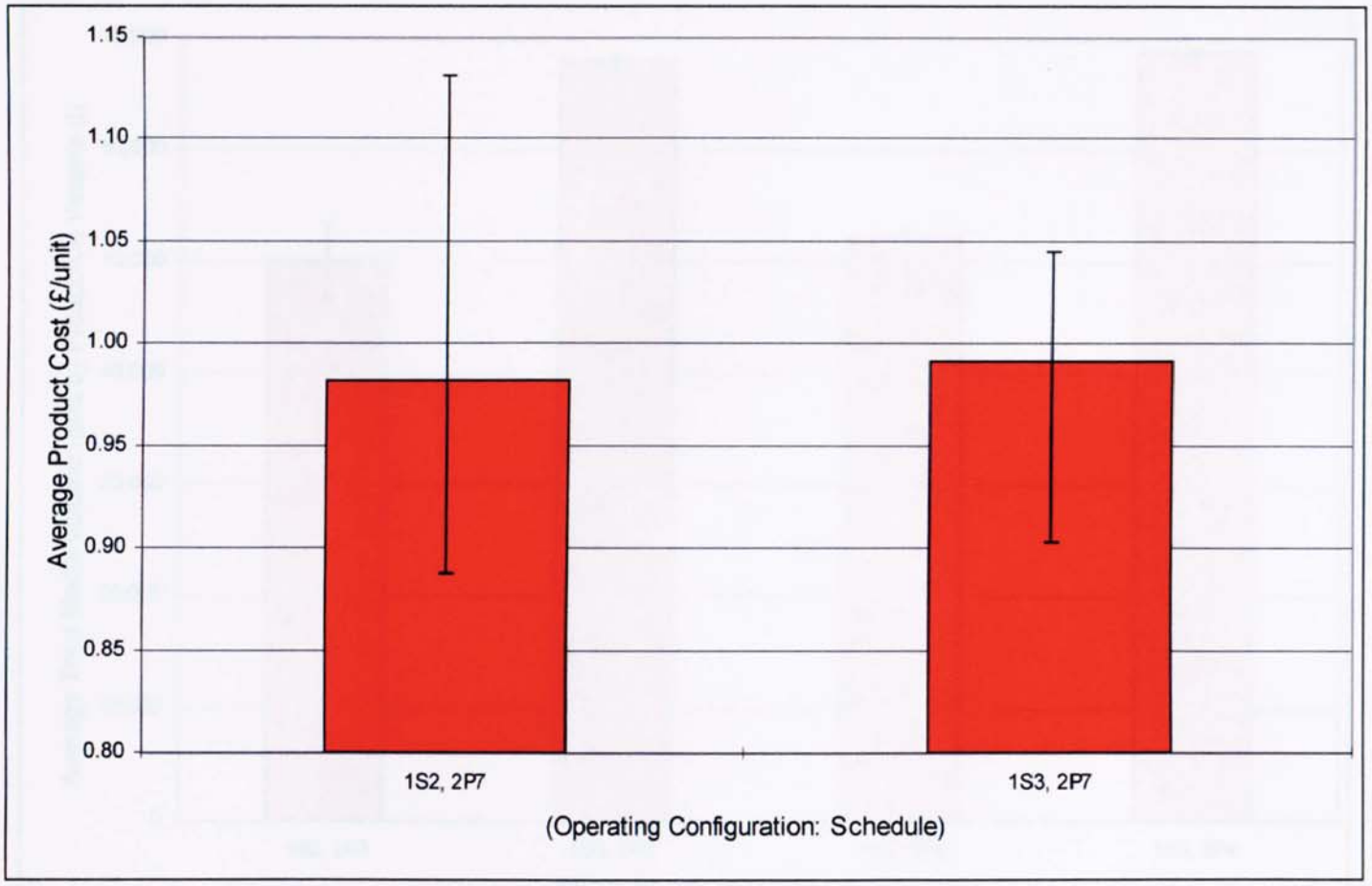

Figure 4.21 A comparison of the average product cost for the 2 Fed-batch operating configuration schedules studied. The average cost based upon the production of 10 complete batches of product. The size of each batch produced during either of the operating schedules will not vary significantly, therefore the profile can then be assumed to represent the average product cost per batch of product.

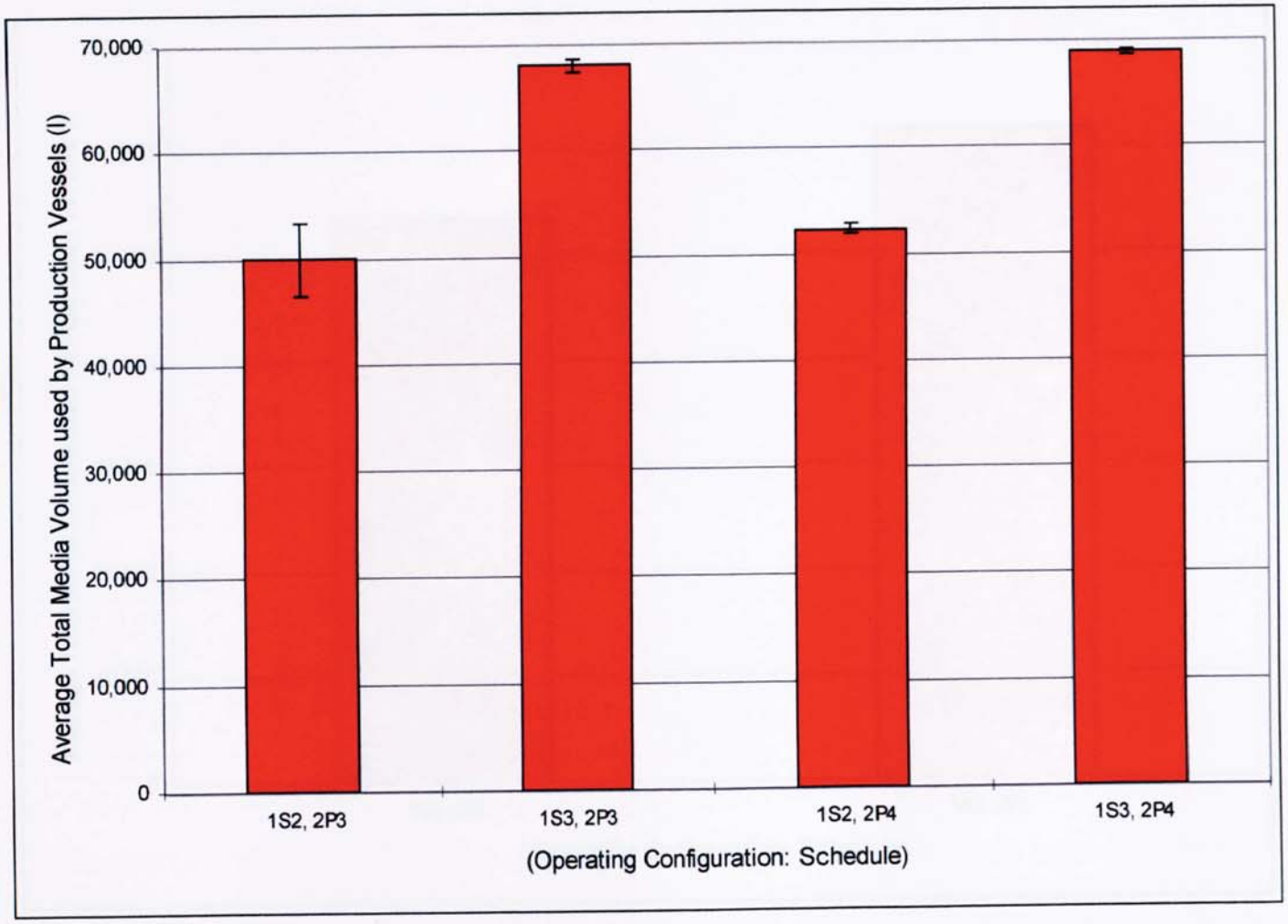

Figure 4.22 Characteristic total average nutrient media volume consumption profile for the Batch operating configuration schedules. Profiles represent only the total average nutrient media used by the production stage vessels in completing a 10 product batch campaign.

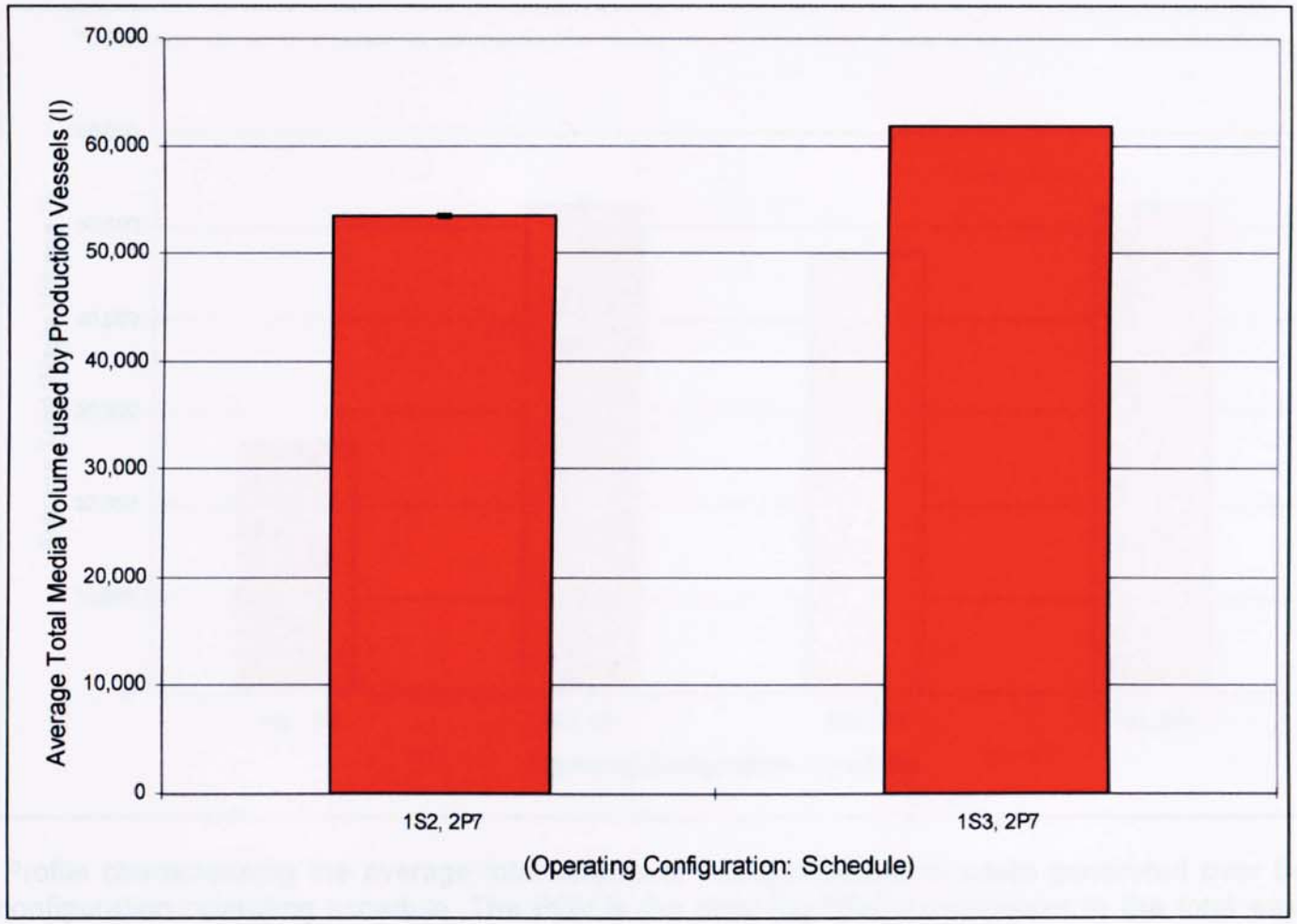

Figure 4.23 Characteristic total average nutrient media volume consumption profile for the Fed-batch operating configuration schedules. Profiles represent only the total average nutrient media used by the production stage vessels in completing a 10 product batch campaign.

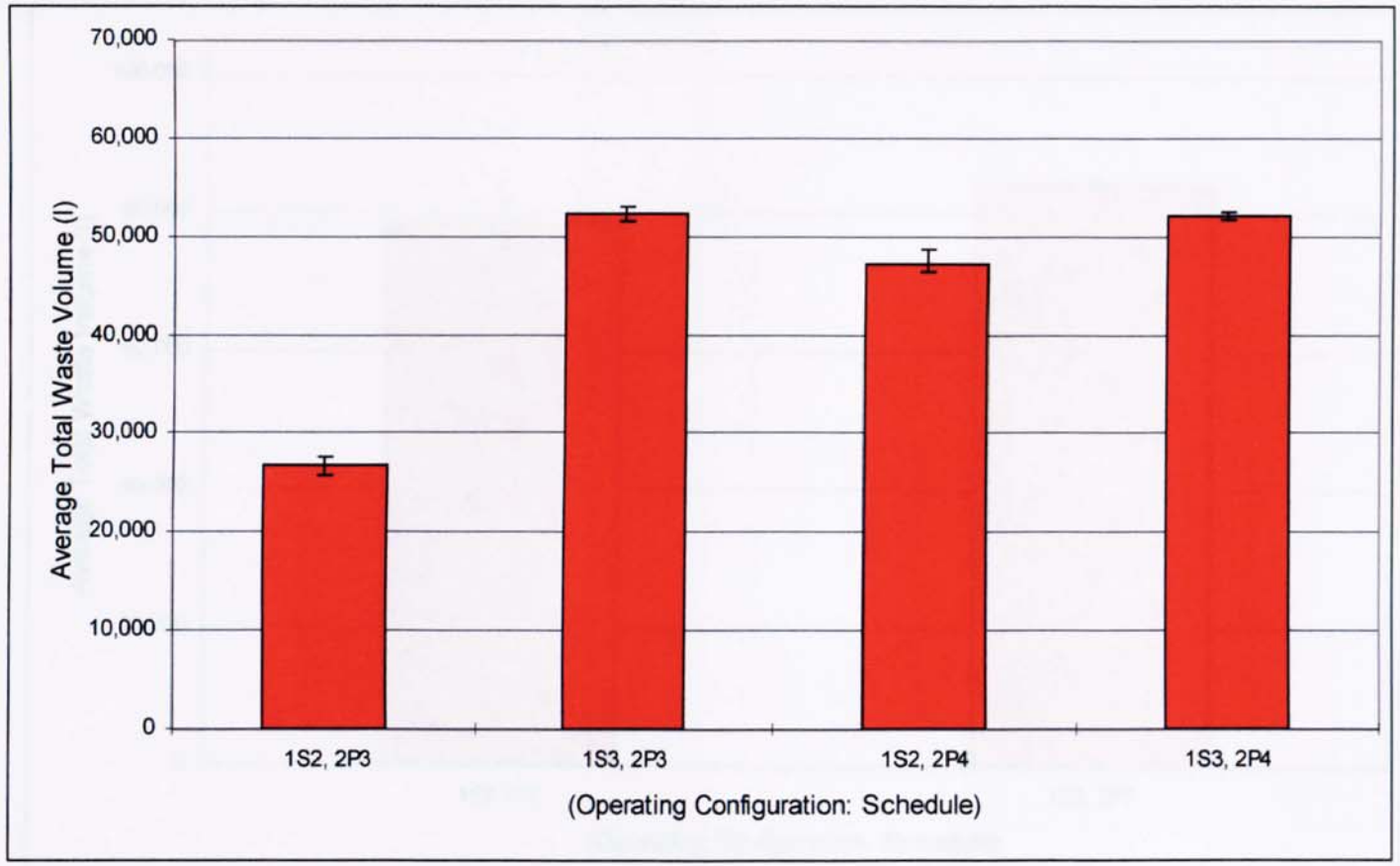

Figure 4.24 Profile characterising the average total volume of biologically active waste generated over the duration of each Batch configuration operating schedule. The PGV is the most significant contributor to the total waste generated and therefore the profile represents the average total waste generated by the PGV. The production vessels make a zero contribution to the total waste volume since the total volume of any production vessel represents the final product batch. Also the production vessels are assumed to complete all batches without any batch failures.

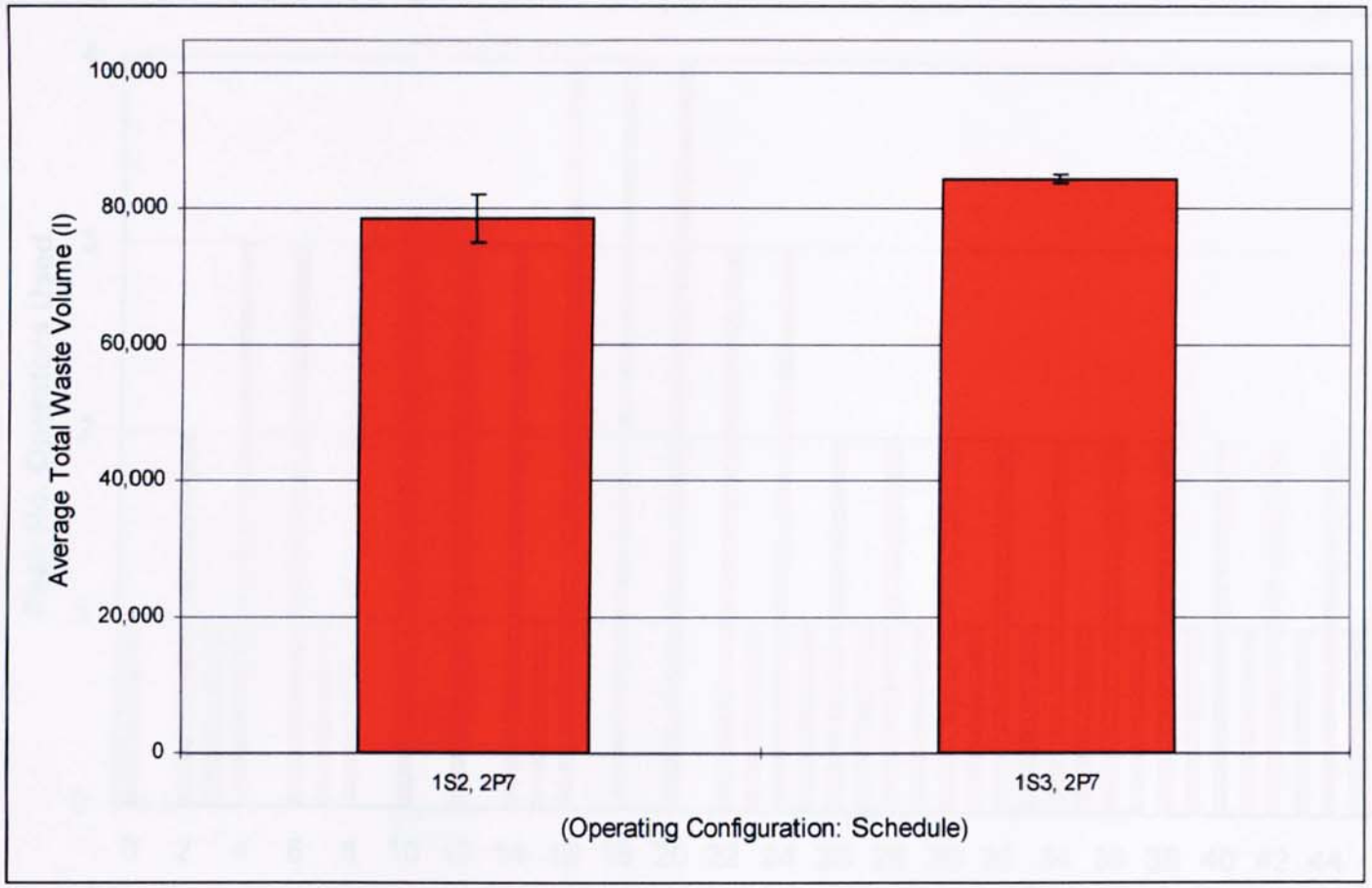

Figure 4.25 Profile characterising the average total volume of biologically active waste generated over the duration of each Fed-batch configuration operating schedule. The PGV is the most significant contributor to the total waste generated and therefore the profile represents the average total waste generated by the PGV. The production vessels make a zero contribution to the total waste volume since the total volume of any production vessel represents the final product batch. Also the production vessels are assumed to complete all batches without any batch failures that would require disposal of that batch to waste.

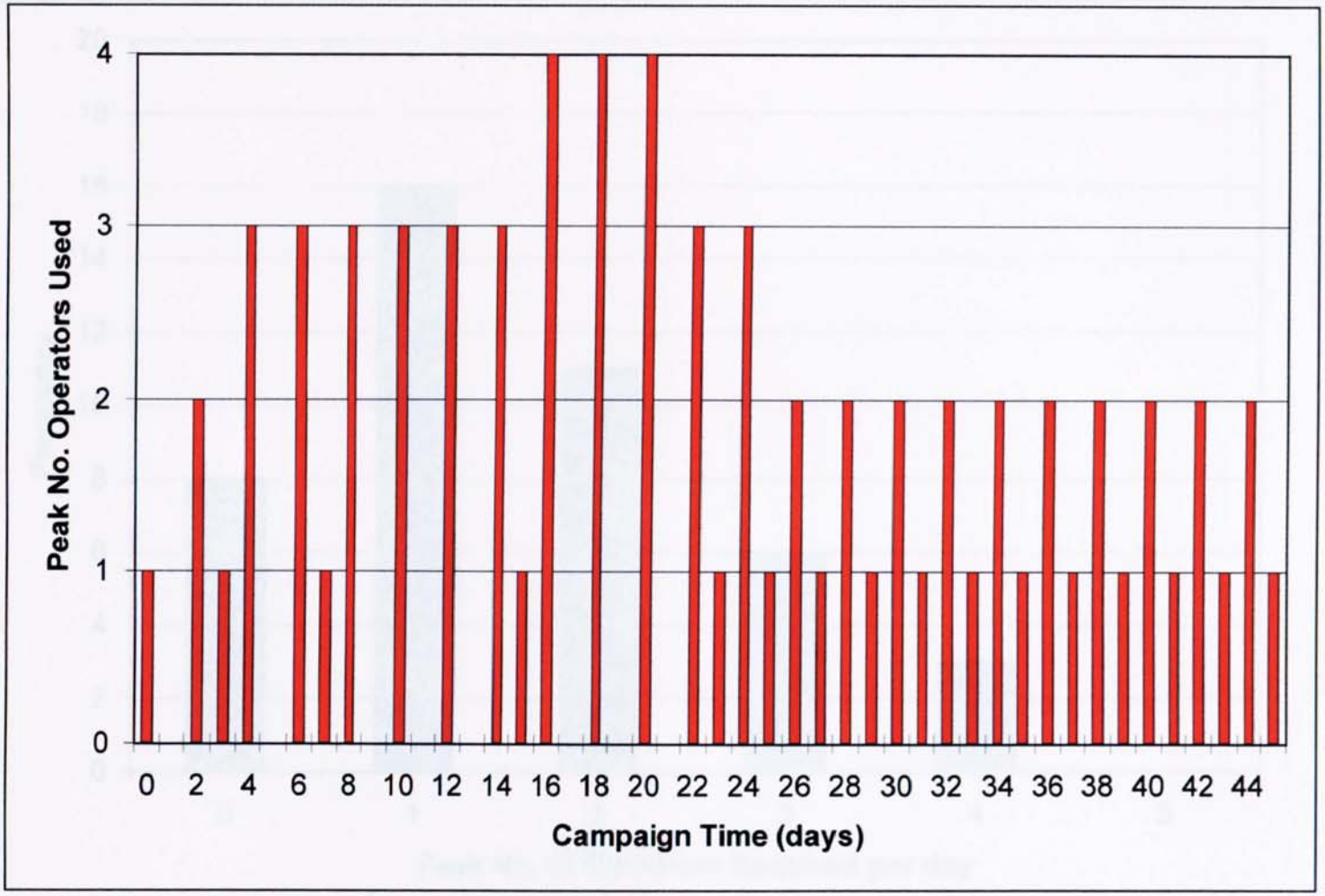

Figure 4.26 Trend chart showing the peak operator requirement or allocation profile resulting from a Batch operating configuration employing the {1S2,2P3} operating schedule. Each peak represents the maximum number of process operators required during that day of operation.

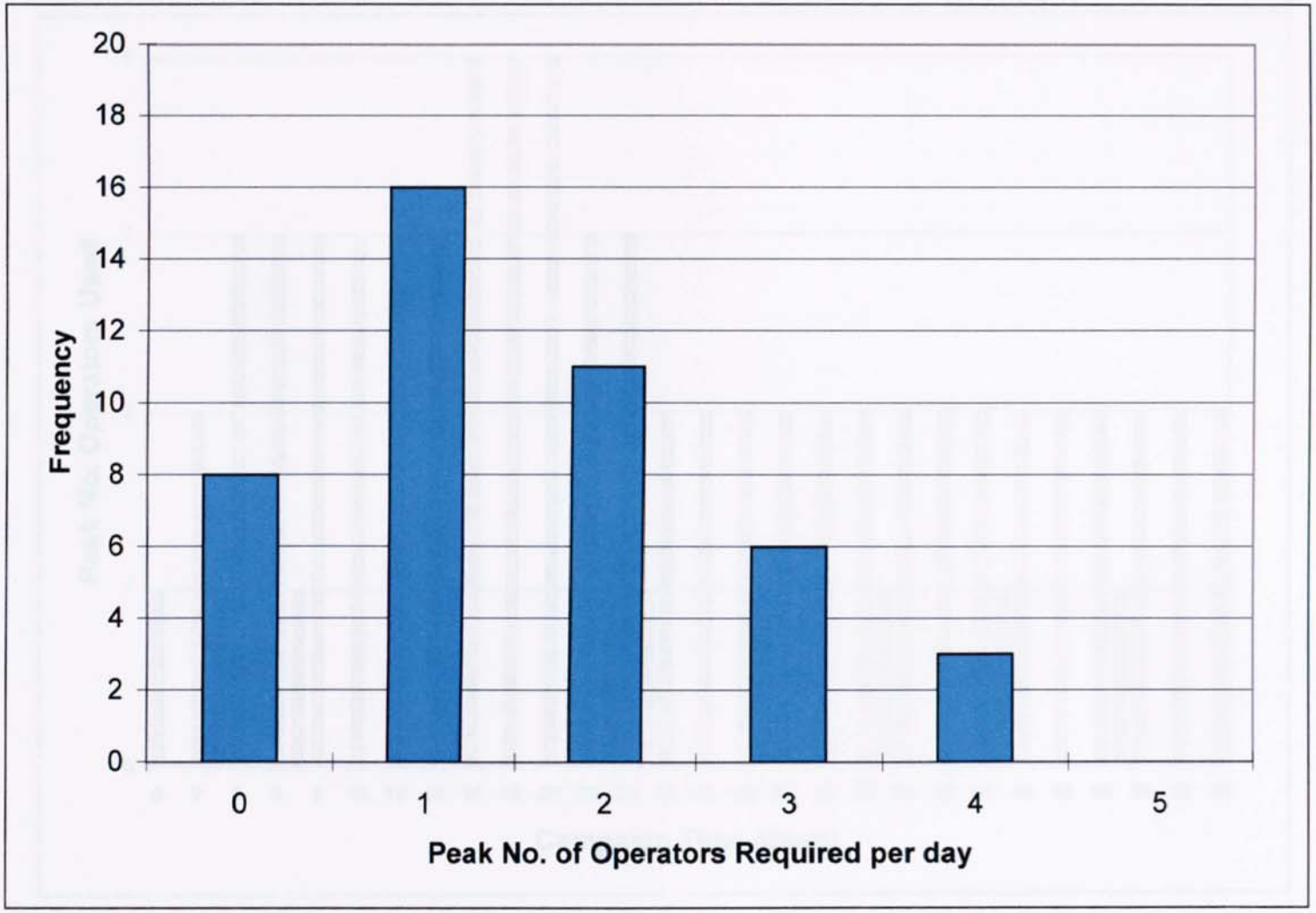

Figure 4.27 Frequency distribution chart showing the relative utilisation of the available operator pool over the entire duration of a batch production campaign employing the {1S2, 2P3} schedule.

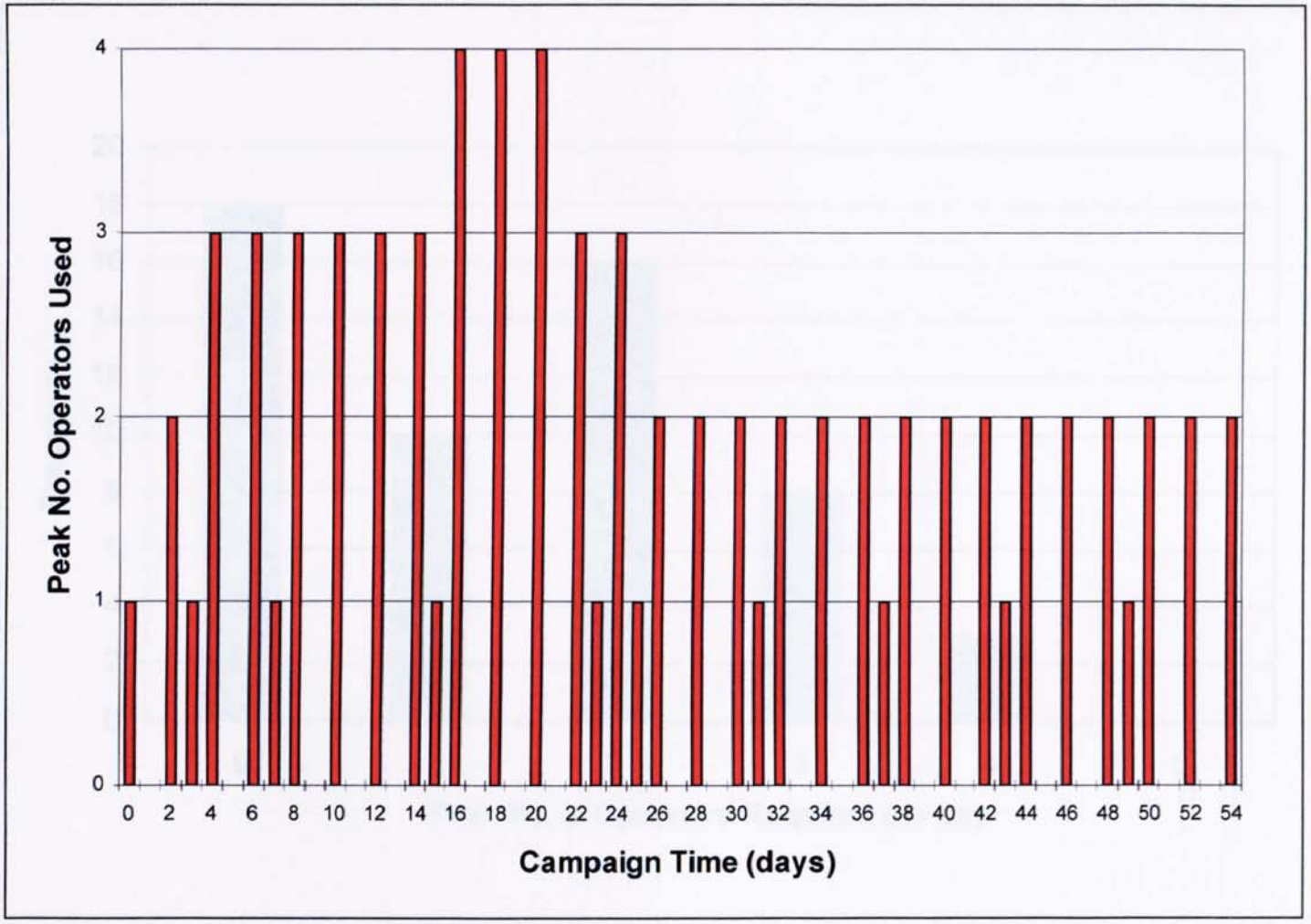

Figure 4.28 Trend chart showing the peak operator requirement or allocation profile resulting from a Batch operating configuration employing the {1S2,2P4} operating schedule. Each peak represents the maximum number of process operators required during that day of operation.

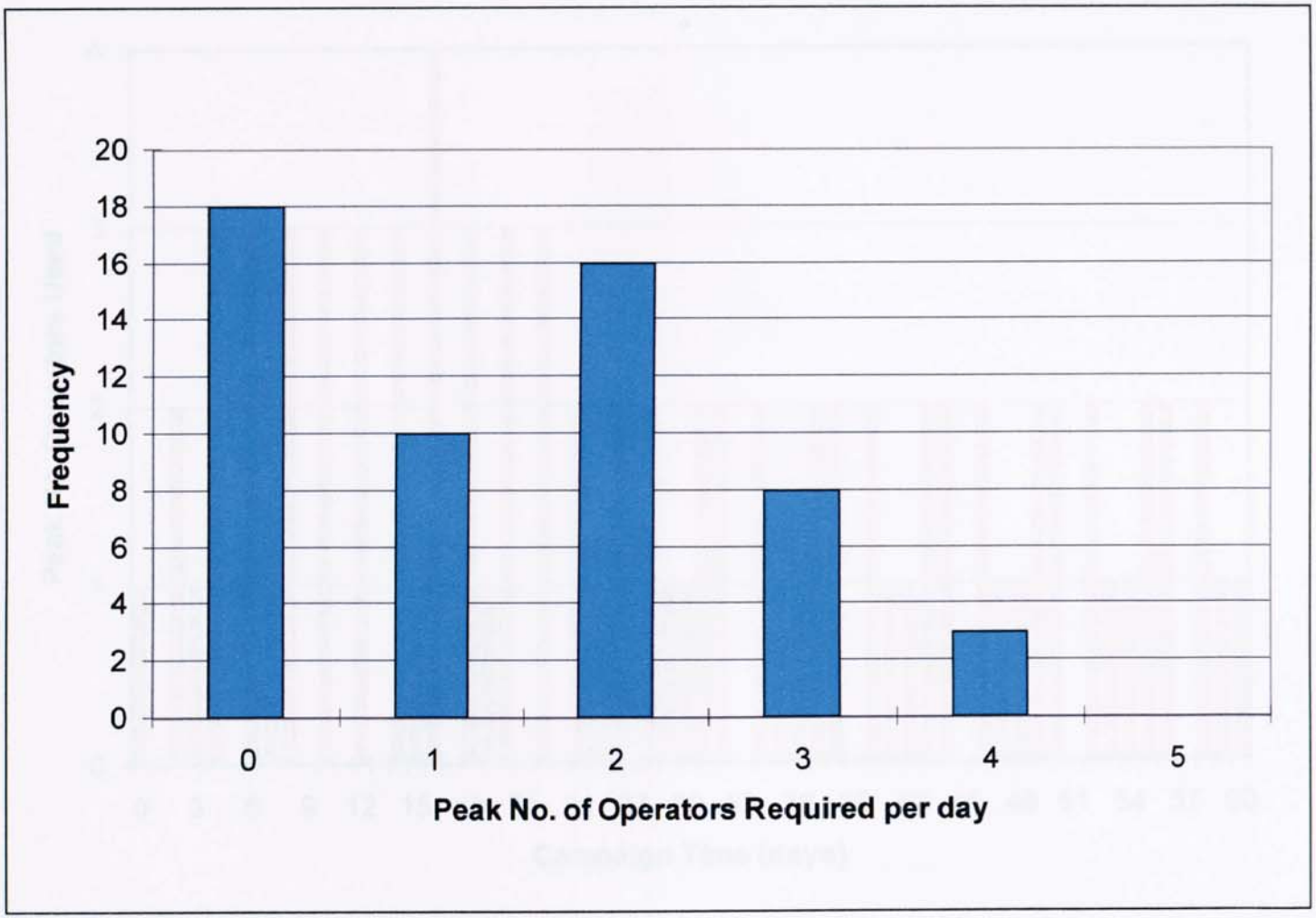

Figure 4.29 Frequency distribution chart showing the relative utilisation of the available operator pool over the entire duration of a batch production campaign employing the {1S2, 2P4} schedule.

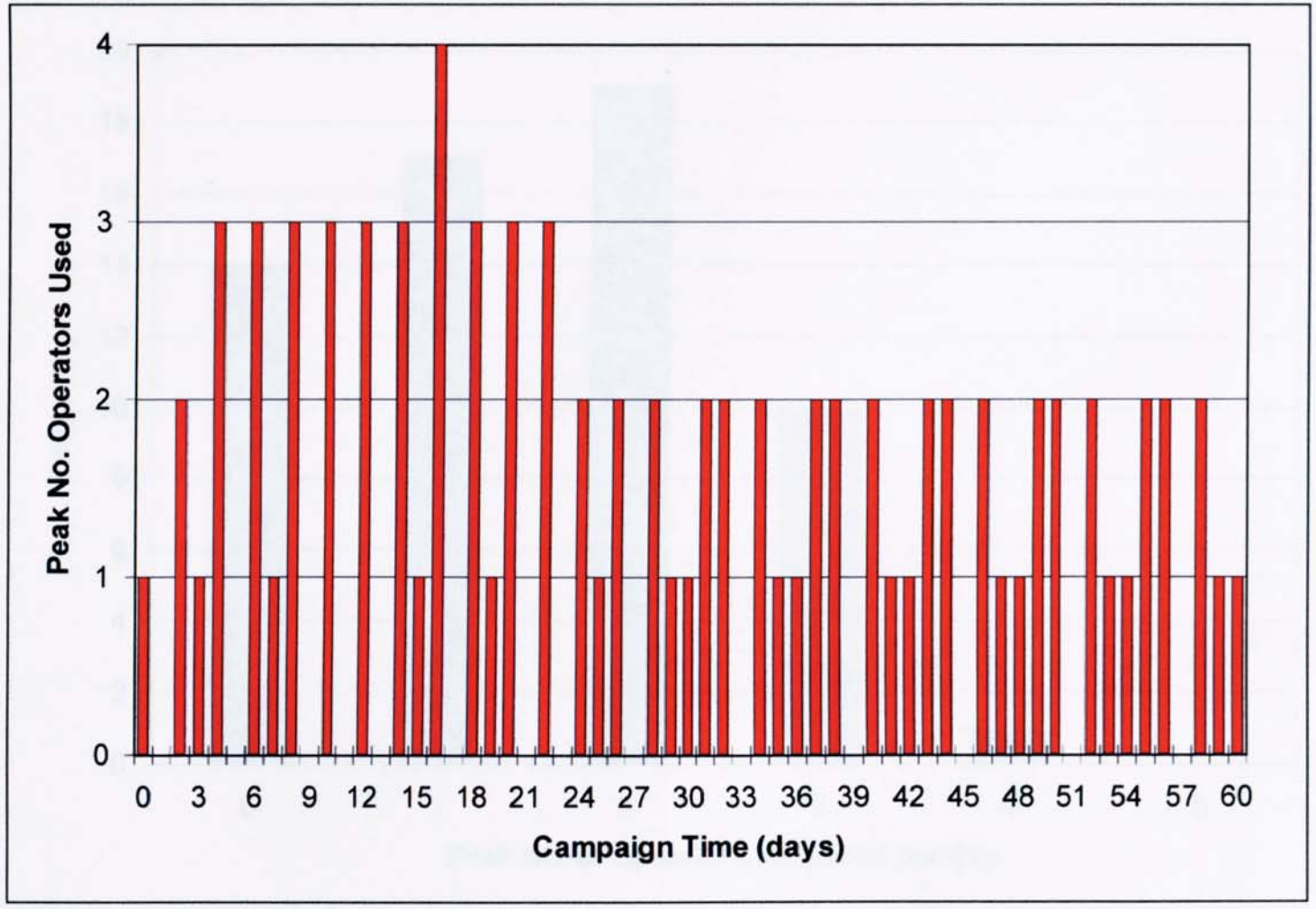

Figure 4.30 Trend chart showing the peak operator requirement or allocation profile resulting from a Batch operating configuration employing the {1S3,2P3} operating schedule. Each peak represents the maximum number of process operators required during that day of operation.

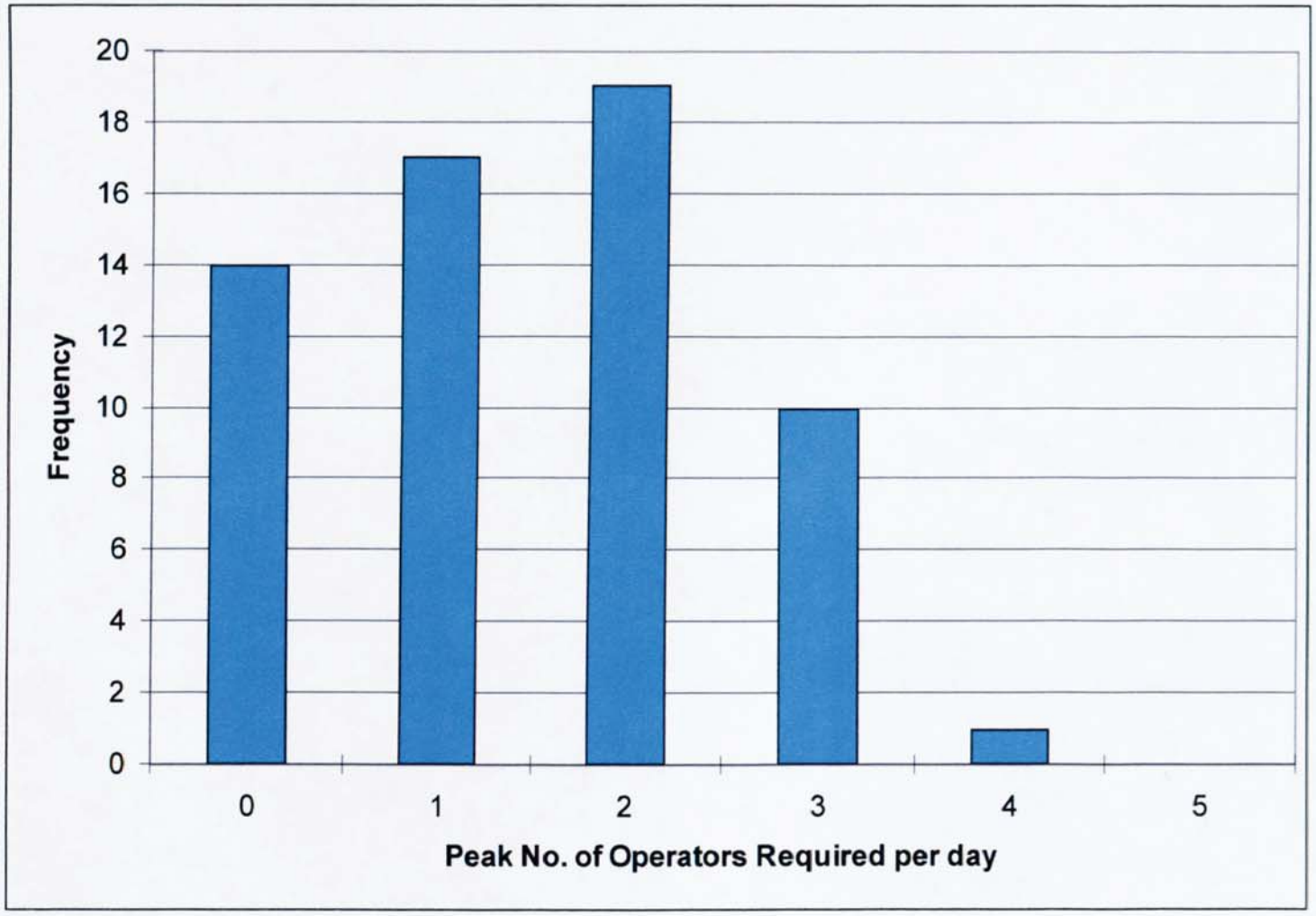

Figure 4.31 Frequency distribution chart showing the relative utilisation of the available operator pool over the entire duration of a batch production campaign employing the {1S3, 2P3} schedule.

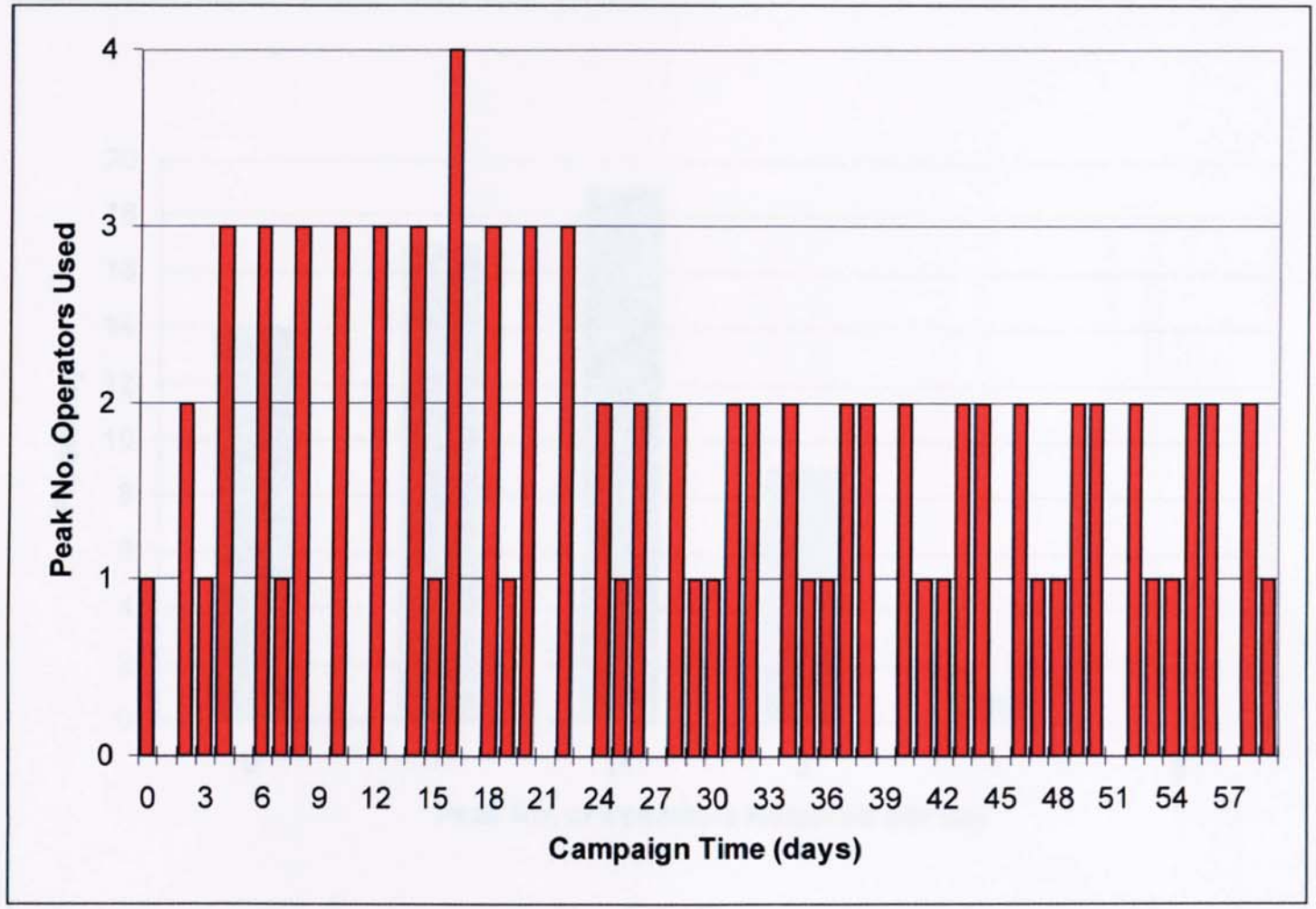

Figure 4.32 Trend chart showing the peak operator requirement or allocation profile resulting from a Batch operating configuration employing the {1S3,2P4} operating schedule. Each peak represents the maximum number of process operators required during that day of operation.

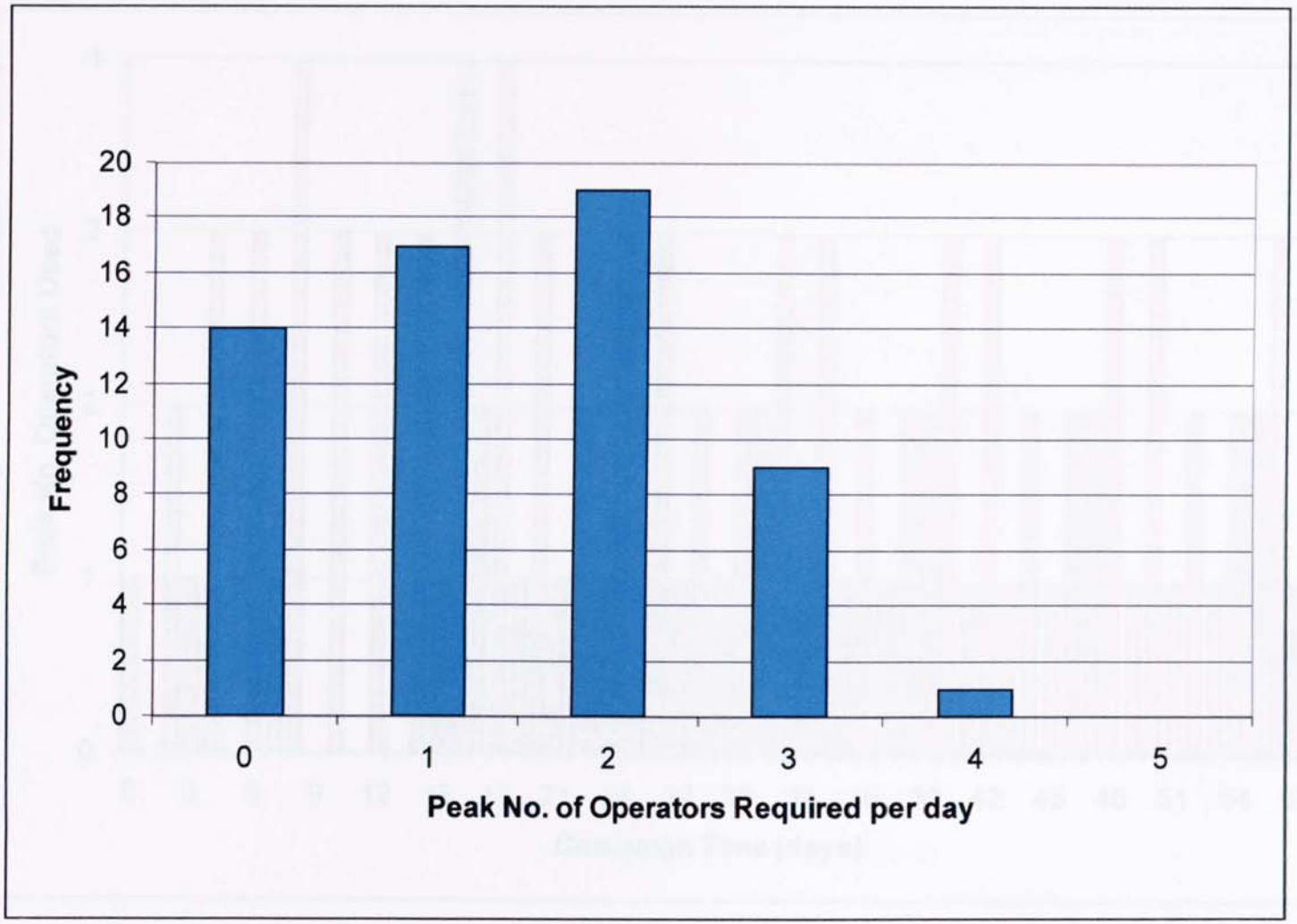

Figure 4.33 Frequency distribution chart showing the relative utilisation of the available operator pool over the entire duration of a batch production campaign employing the {1S3, 2P4} schedule.

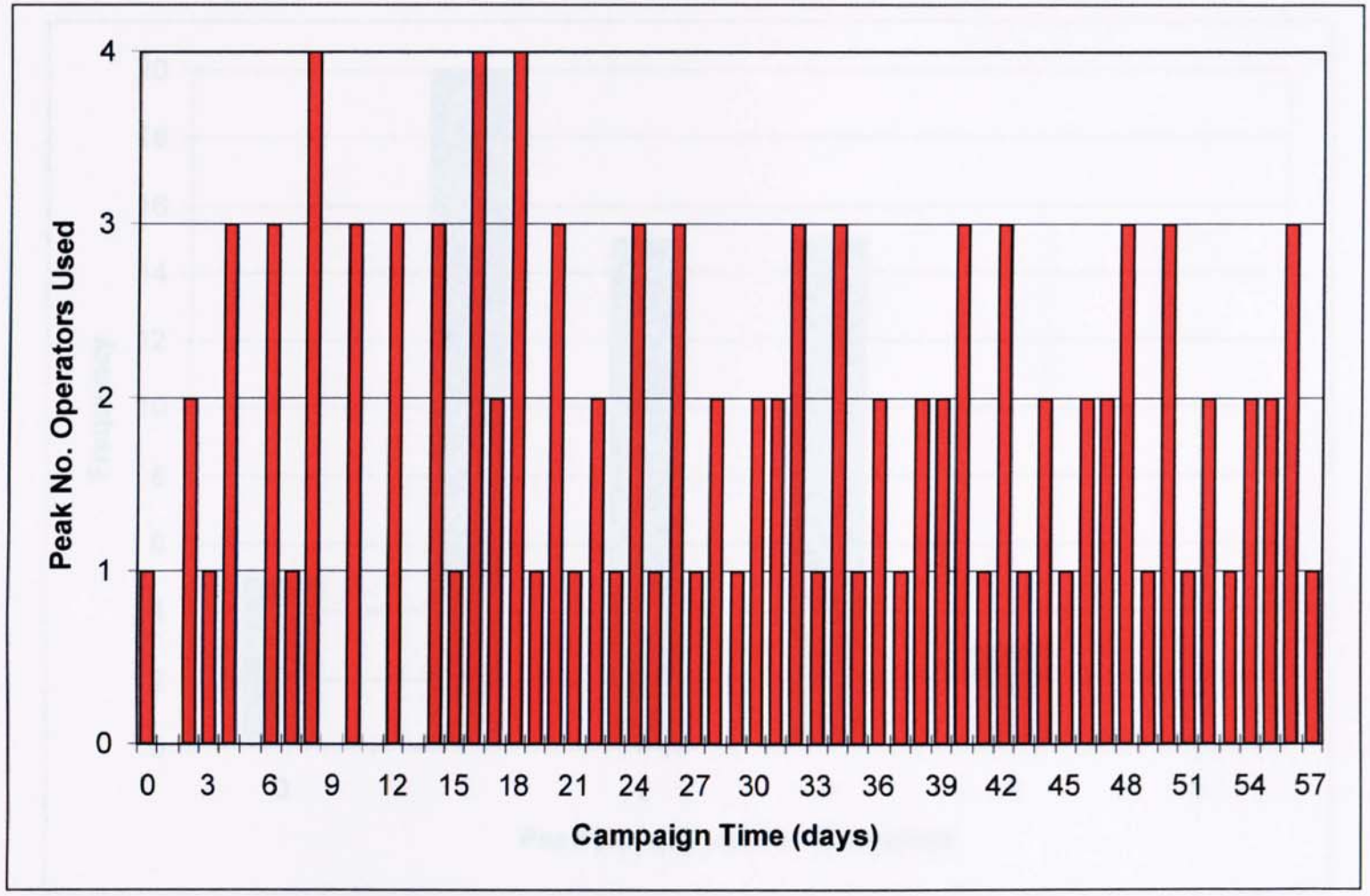

Figure 4.34 Trend chart showing the peak operator requirement or allocation profile resulting from a Batch operating configuration employing the {1S2,2P7} operating schedule. Each peak represents the maximum number of process operators required during that day of operation.

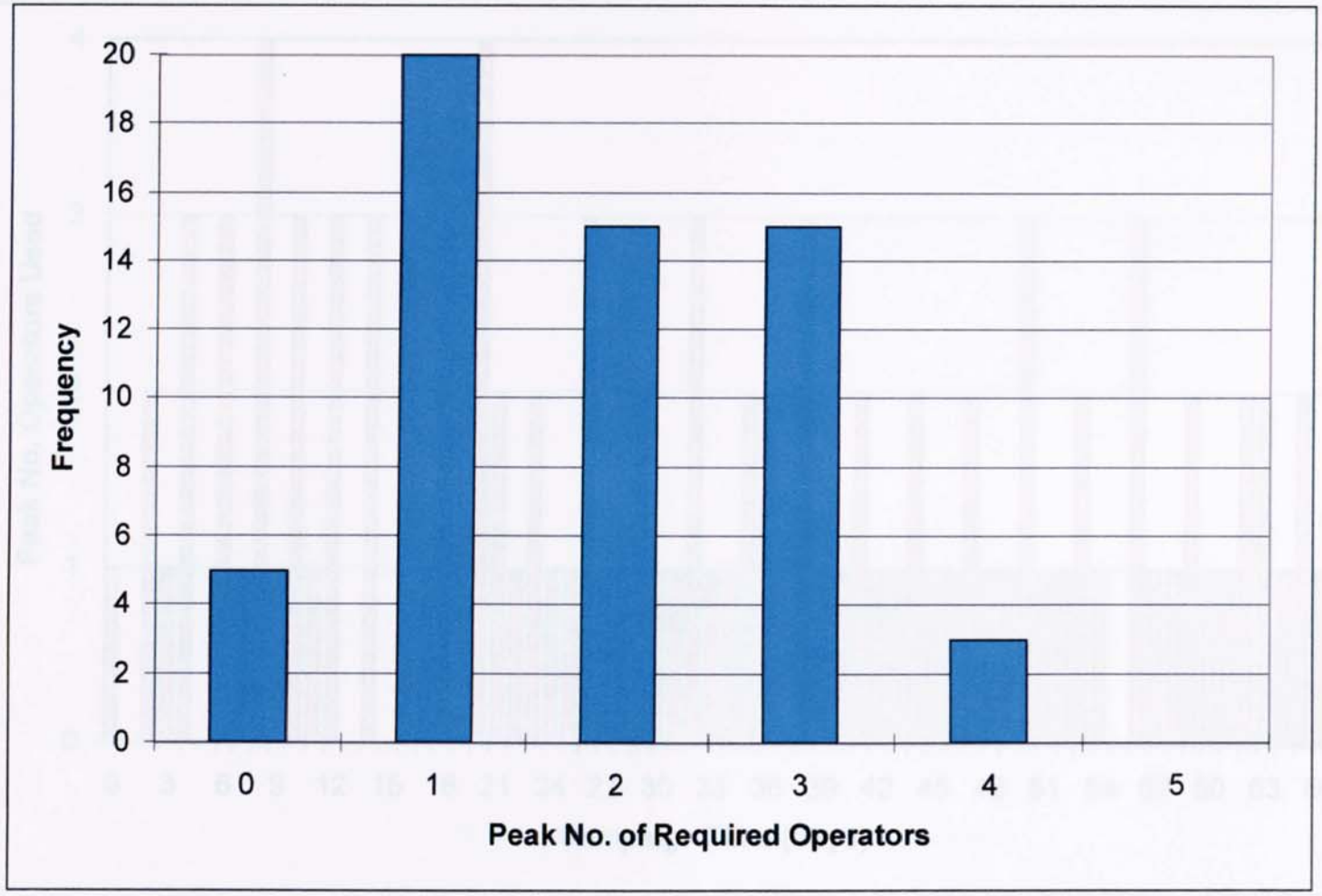

Figure 4.35 Frequency distribution chart showing the relative utilisation of the available operator pool over the entire duration of a batch production campaign employing the {1S2, 2P7} schedule.

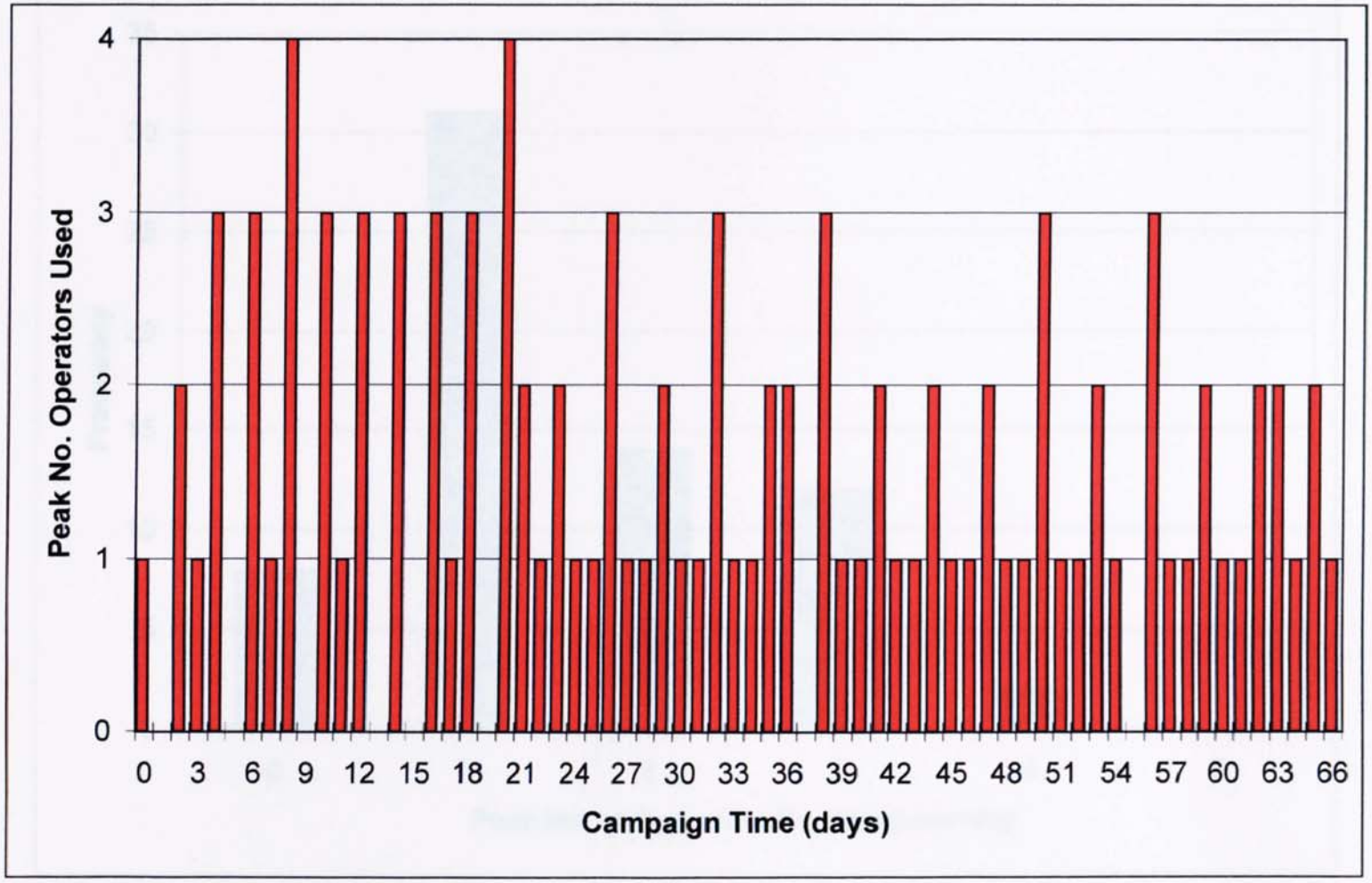

Figure 4.36 Trend chart showing the peak operator requirement or allocation profile resulting from a Batch operating configuration employing the {1S3,2P7} operating schedule. Each peak represents the maximum number of process operators required during that day of operation.

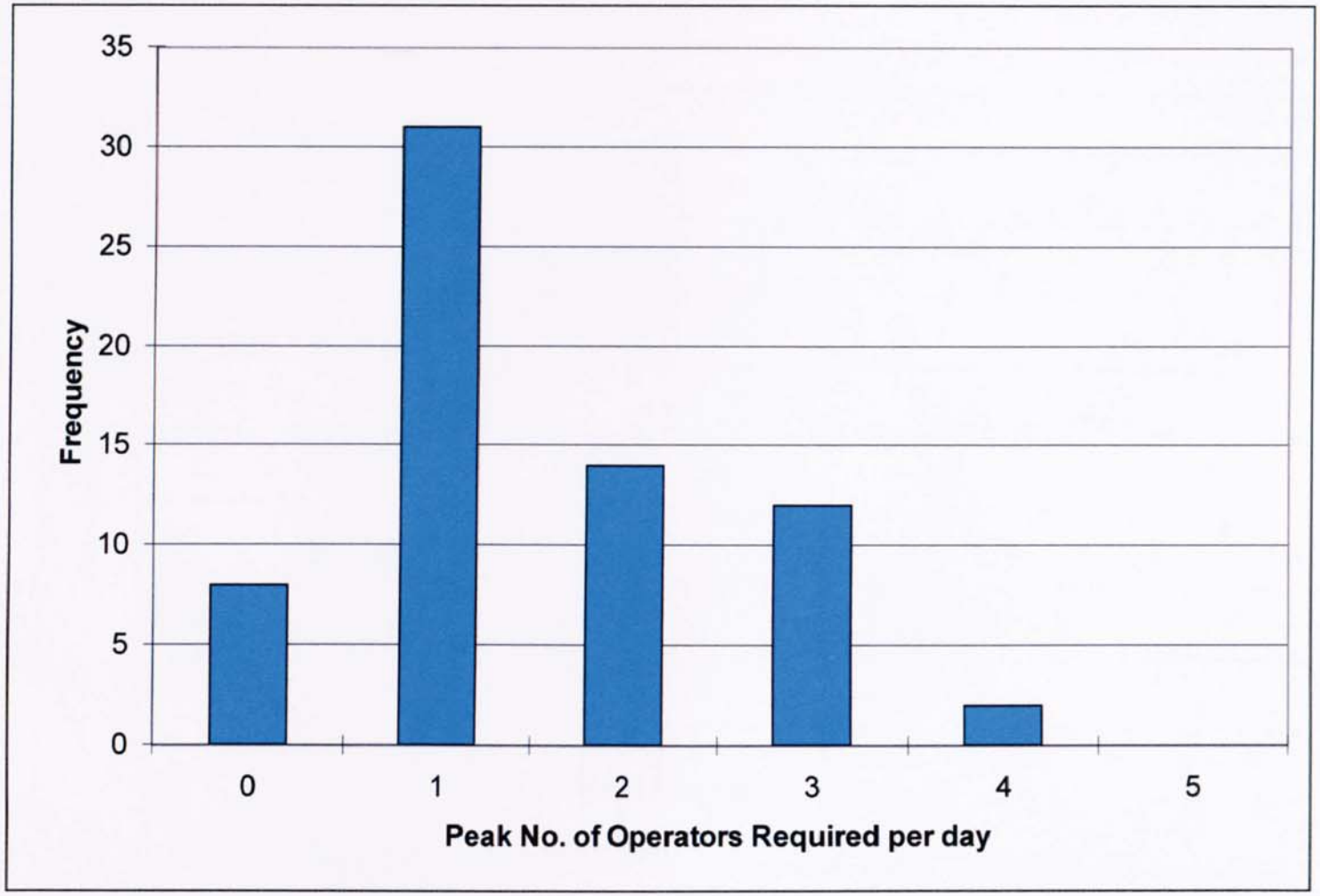

Figure 4.37 Frequency distribution chart showing the relative utilisation of the available operator pool over the entire duration of a batch production campaign employing the {1S3, 2P7} schedule.

APPENDIX D (Chapter V Figures)

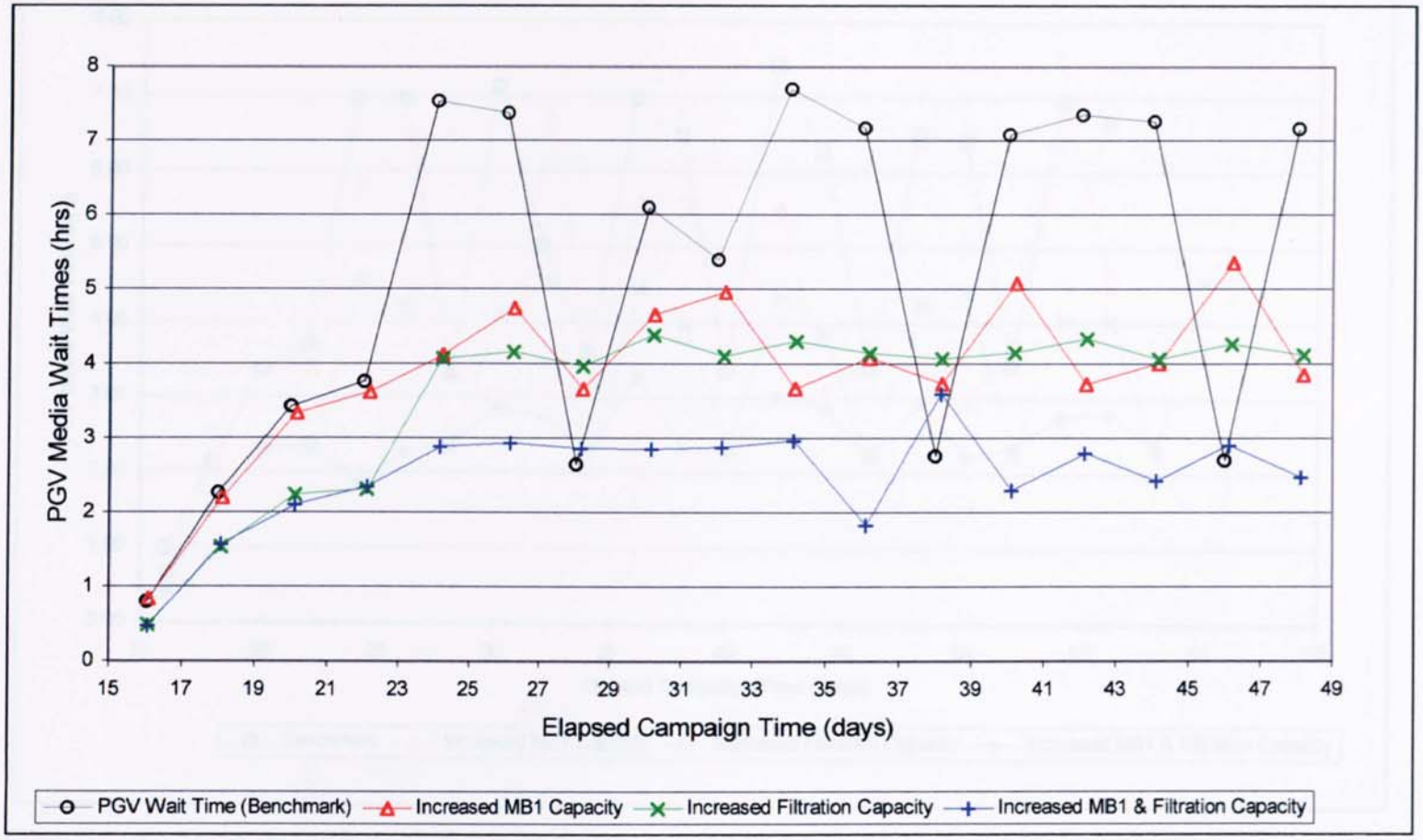

Figure 5.1 Chart showing the effect of increasing the media preparation capacity and the media filtration rate upon the media waiting times experienced by the PGV using the Batch {1S2, 2P3} schedule

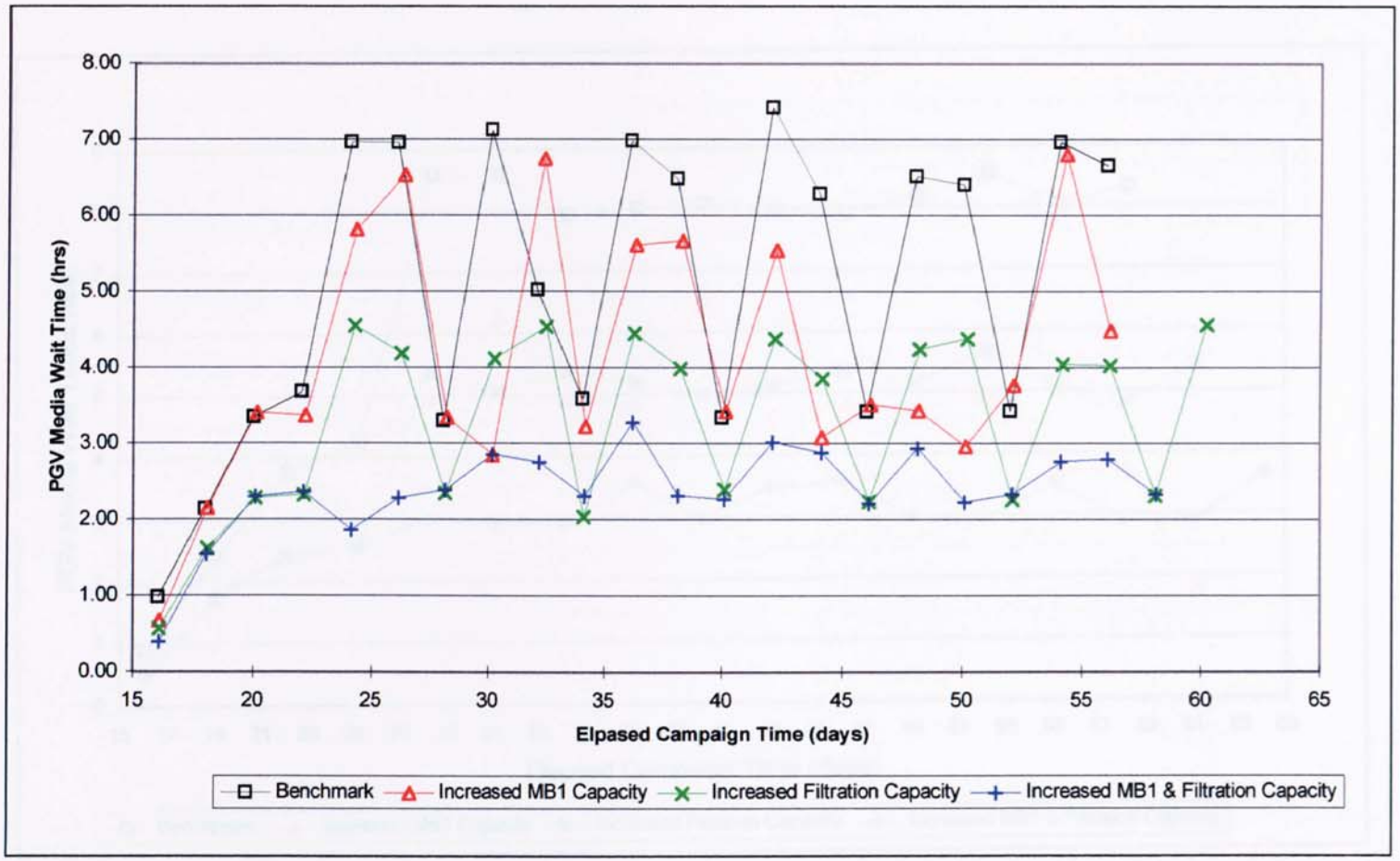

Figure 5.2 Chart showing the effect of increasing the media preparation capacity and the media filtration rate upon the media waiting times experienced by the PGV using the Batch {1S2, 2P4} schedule.

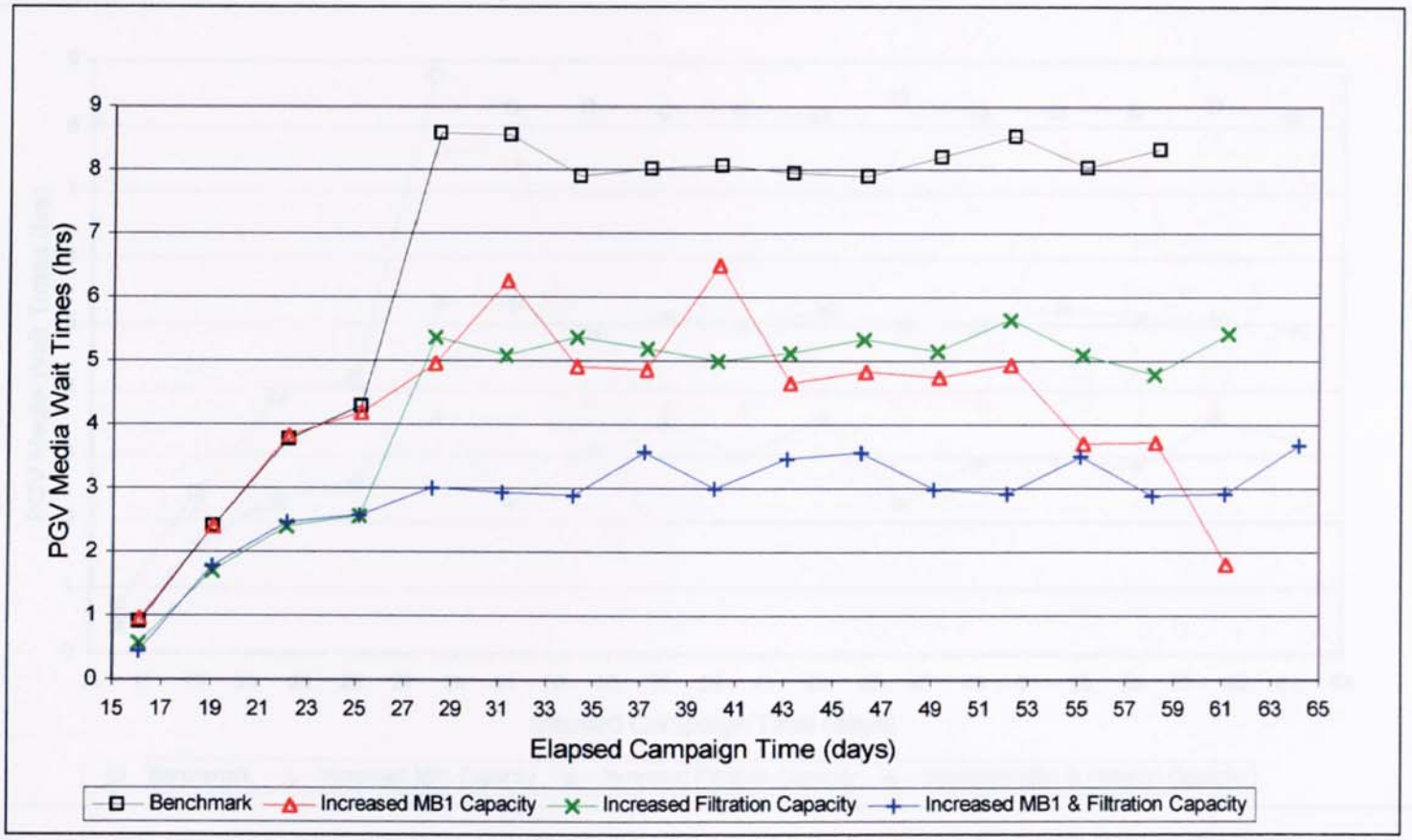

Figure 5.3 Chart showing the effect of increasing the media preparation capacity and the media filtration rate upon the media waiting times experienced by the PGV using the Batch {1S3, 2P3} schedule.

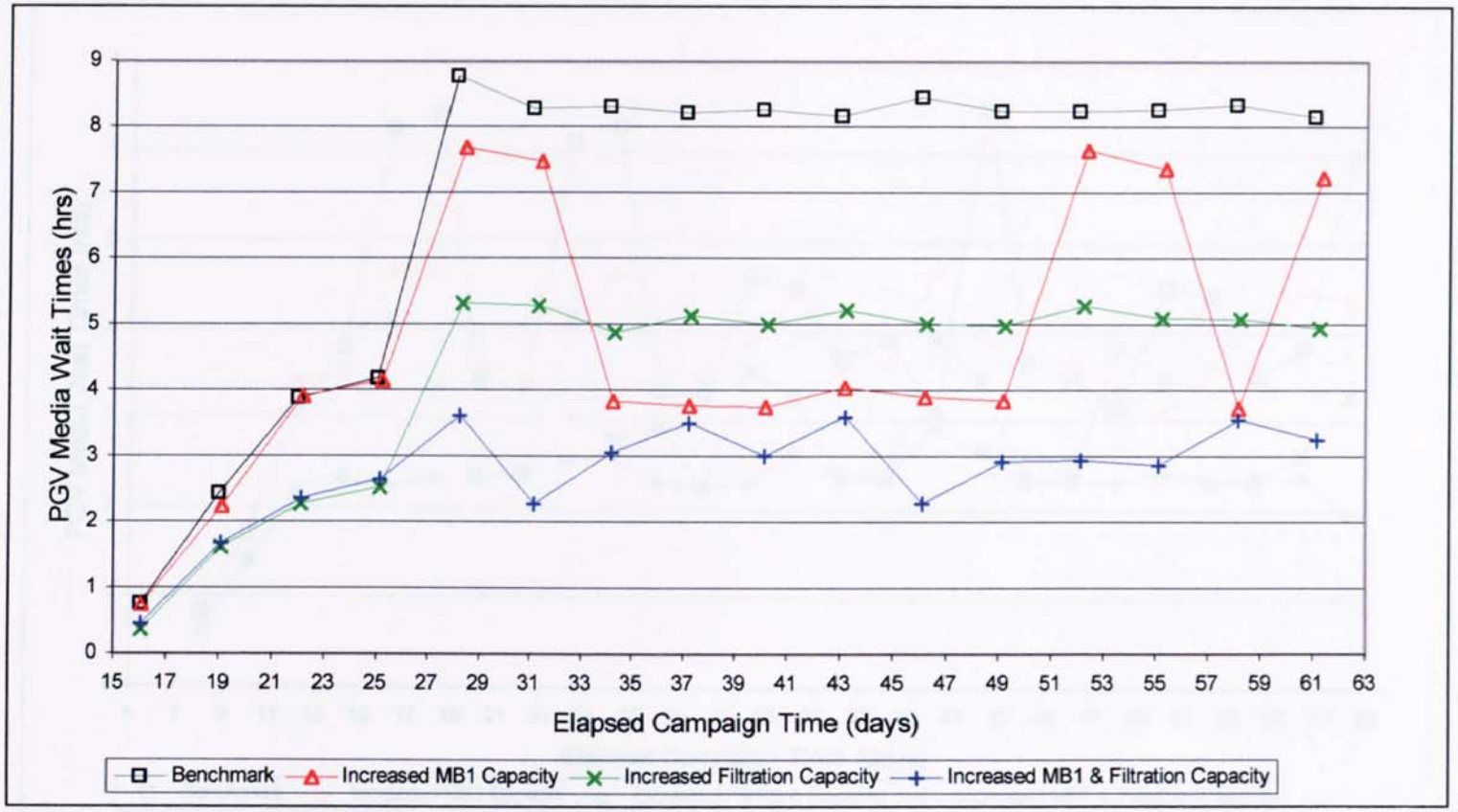

Figure 5.4 Chart showing the effect of increasing the media preparation capacity and the media filtration rate upon the media waiting times experienced by the PGV using the Batch {1S3, 2P4} schedule.

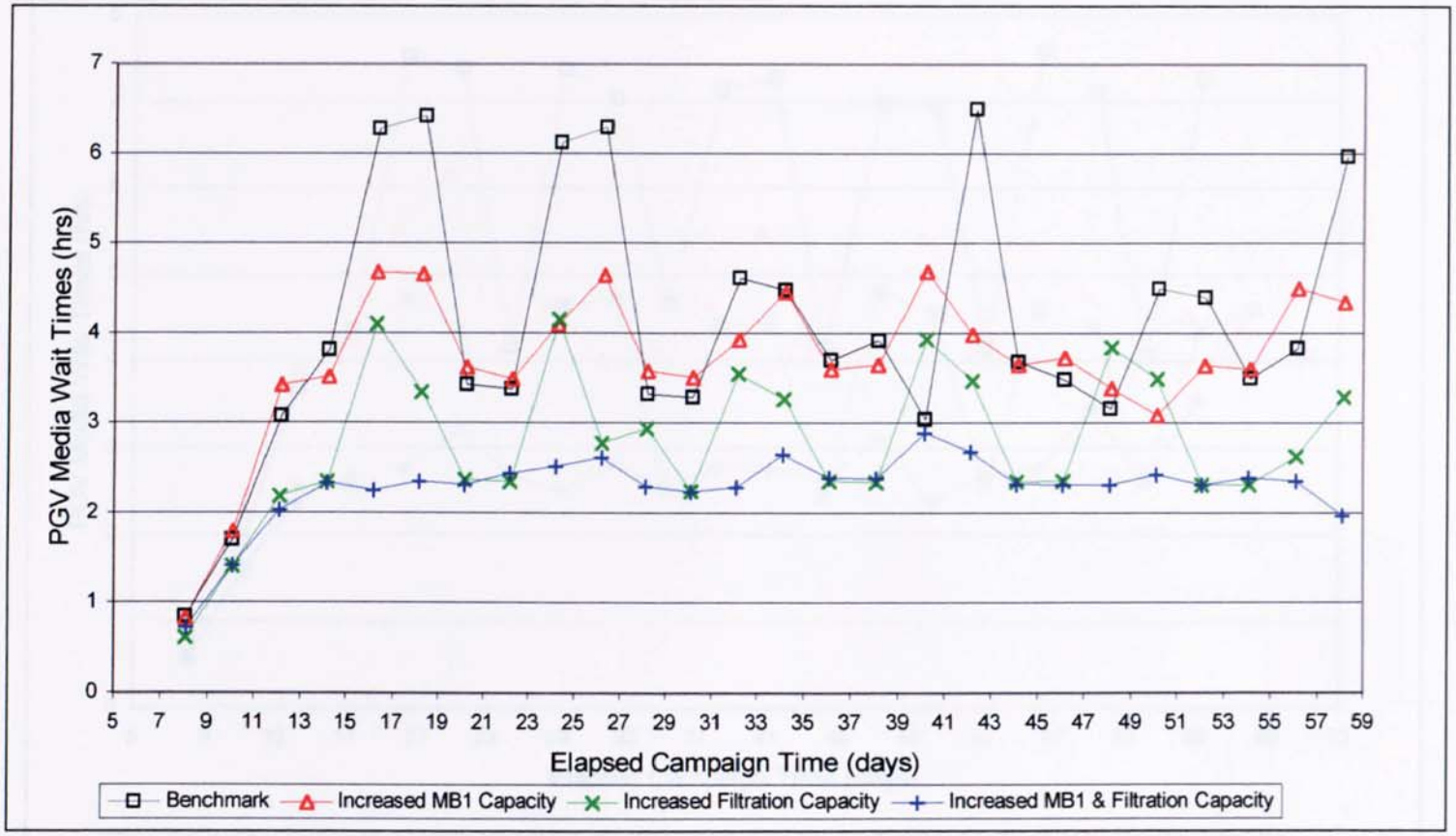

Figure 5.5 Chart showing the effect of increasing the media preparation capacity and the media filtration rate upon the media waiting times experienced by the PGV using the Fed-batch {1S2, 2P7} schedule.

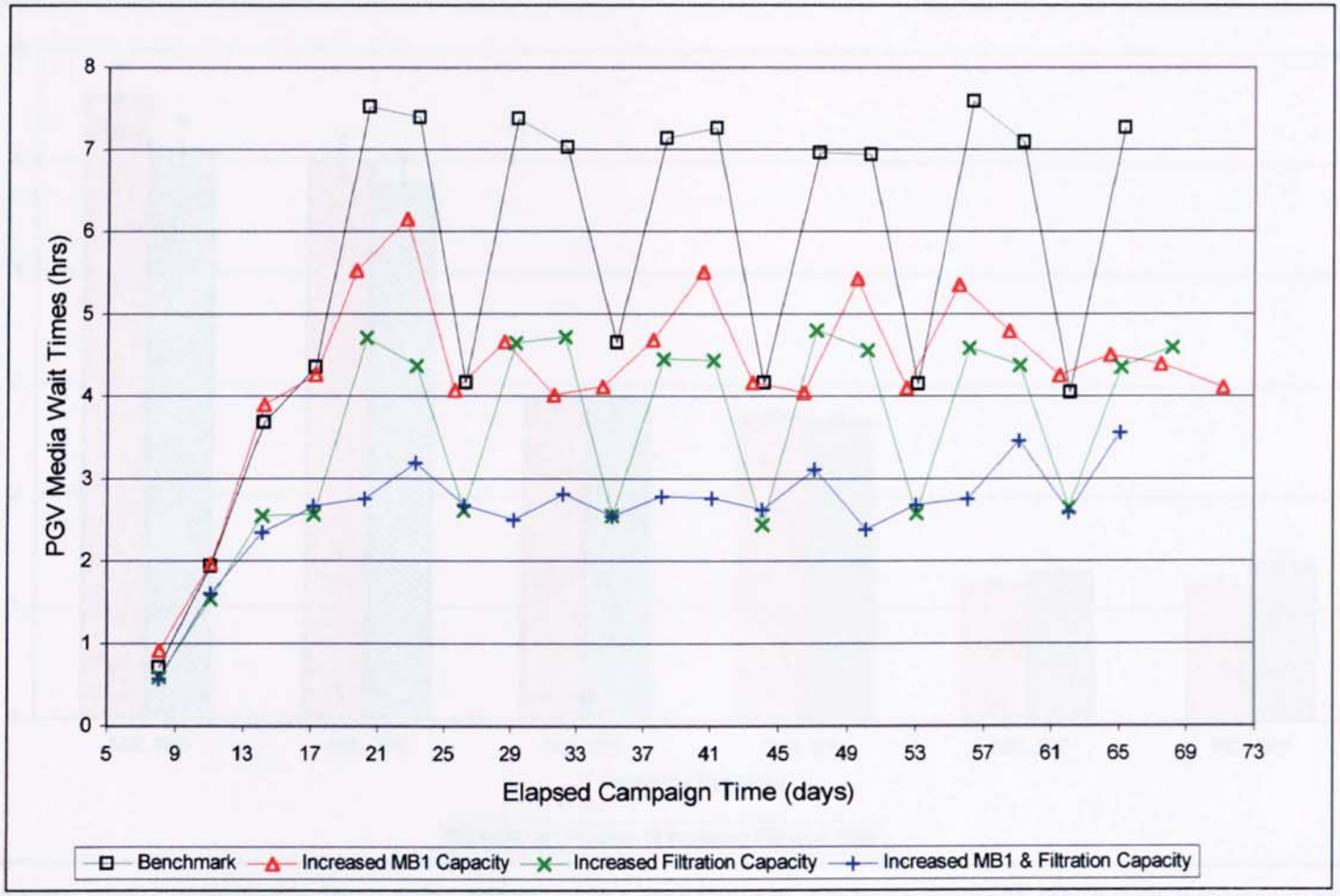

Figure 5.6 Chart showing the effect of increasing the media preparation capacity and the media filtration rate upon the media waiting times experienced by the PGV using the Fed-batch {1S3, 2P7} schedule.

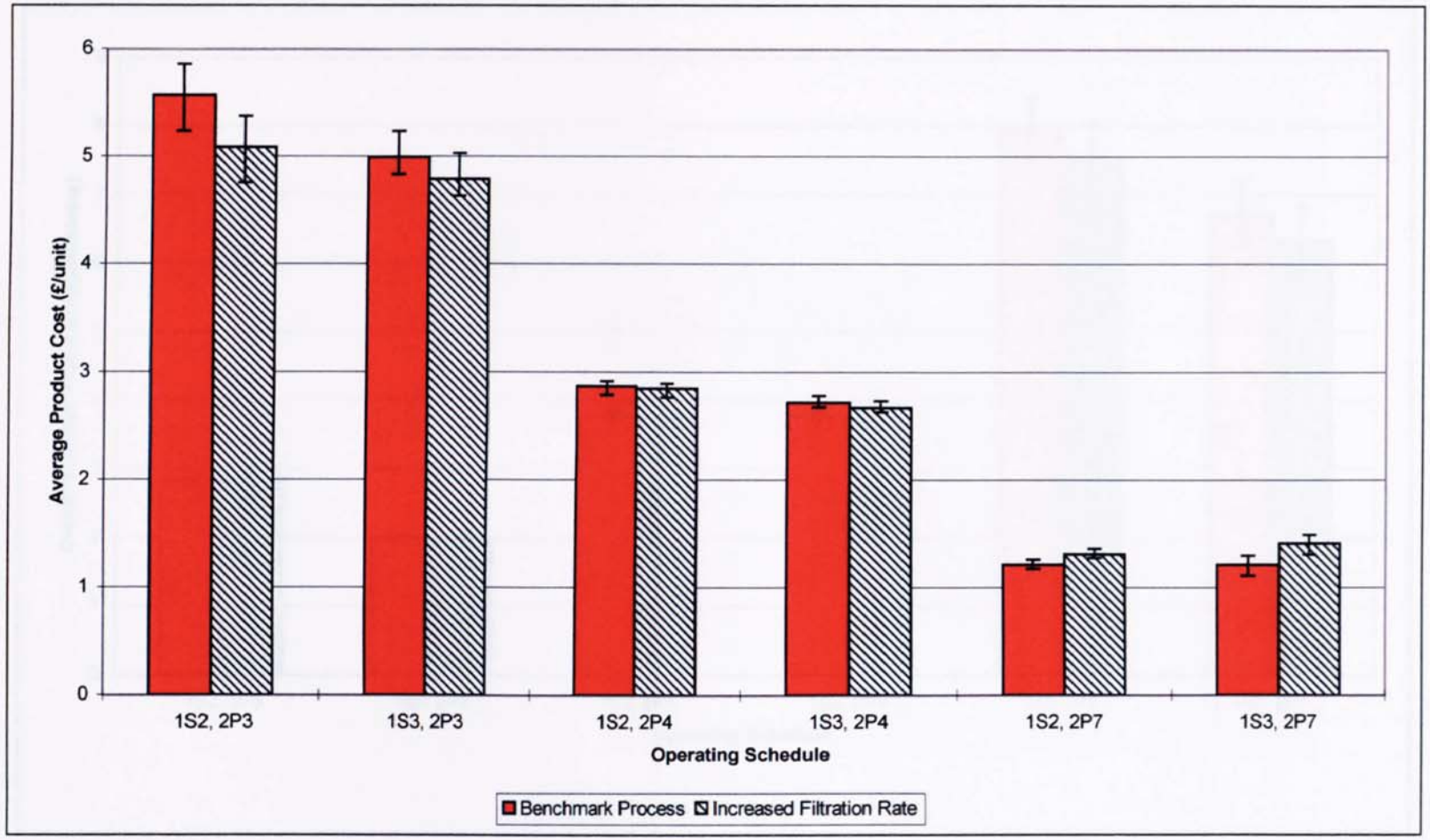

Figure 5.7 Summary chart comparing the performance of the benchmark process against the same process with an increased media filter unit filtration rate. The performance parameter in this case being the average product cost for each operating schedule.

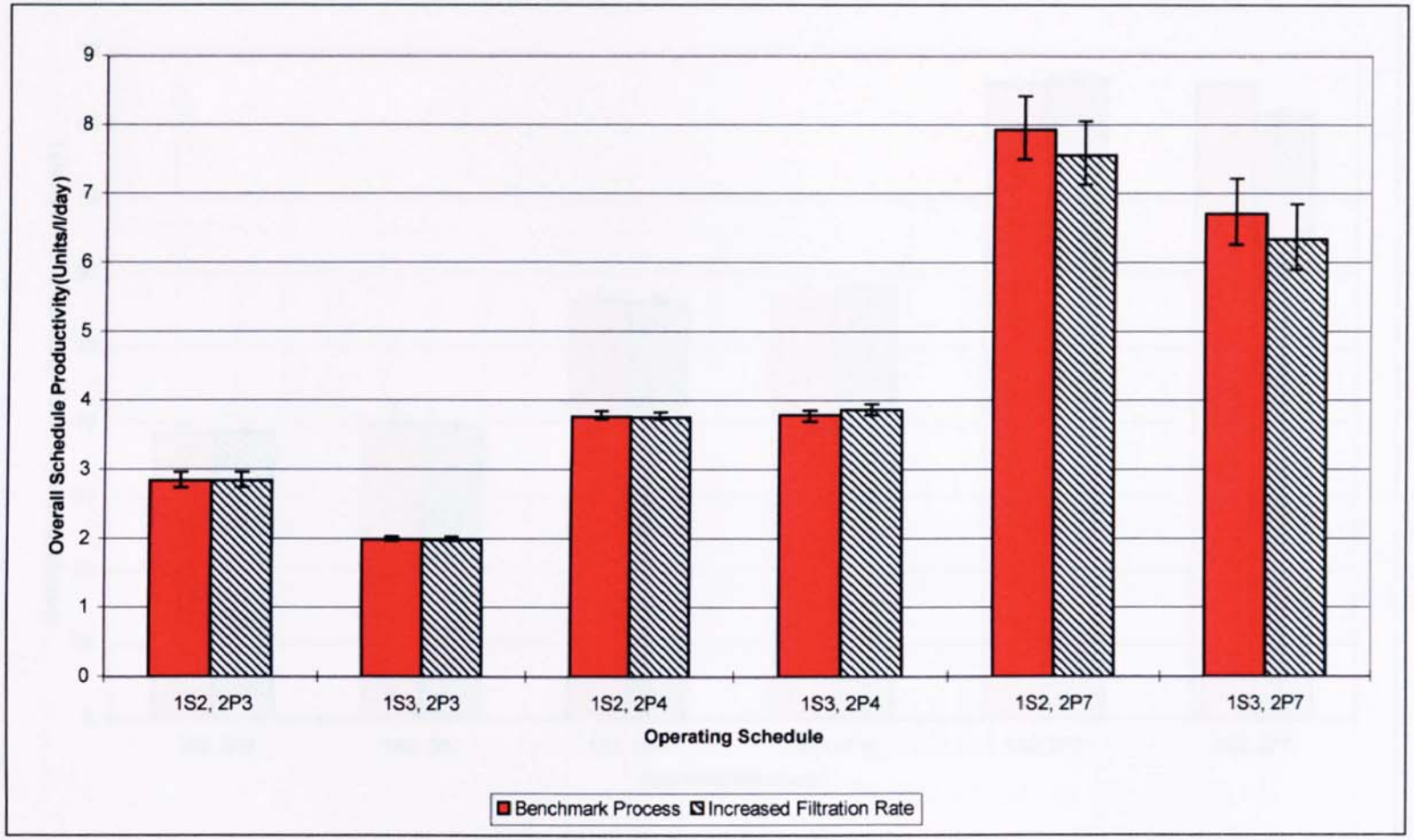

Figure 5.8 Summary chart comparing the overall schedule productivity of the benchmark process against the same process with an increased media filter unit filtration rate.

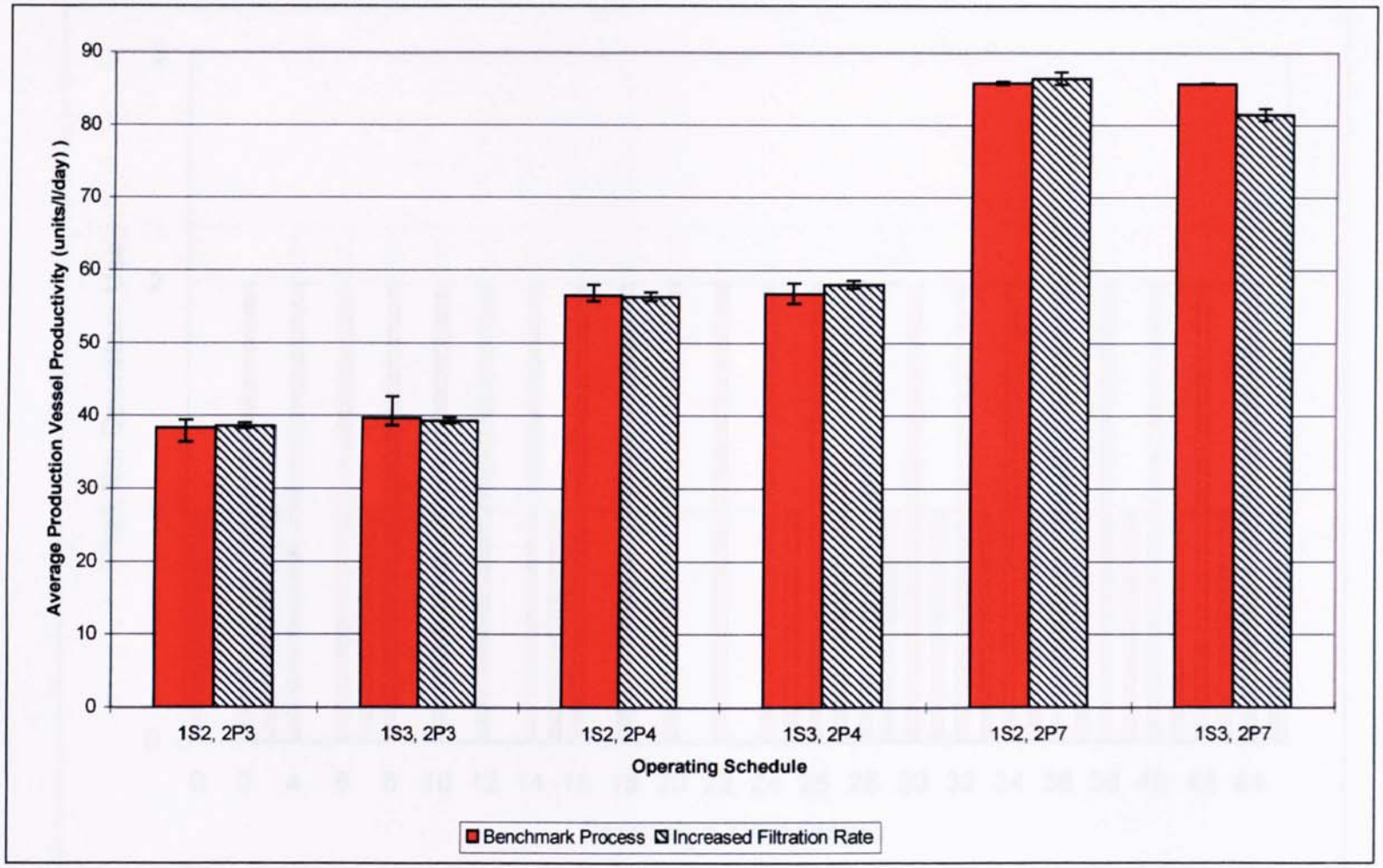

Figure 5.9 Profile comparing the average production vessel productivity attained over each benchmark schedule with the same schedules using an increased media filter unit filtration rate.

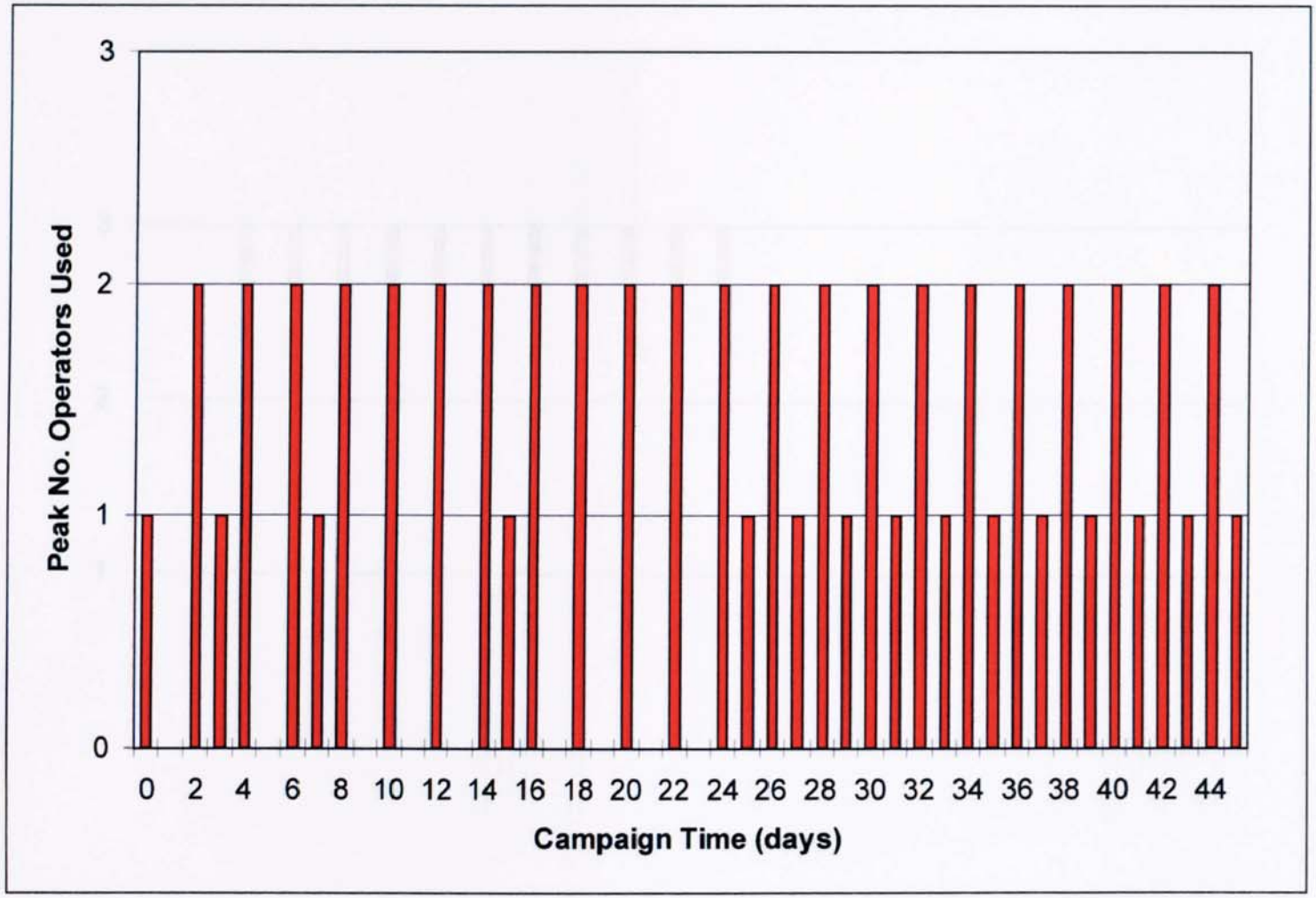

Figure 5.10 Trend chart showing the peak daily operator requirement profile for the {1S2, 2P3} operating schedule with an operator pool size of 2.

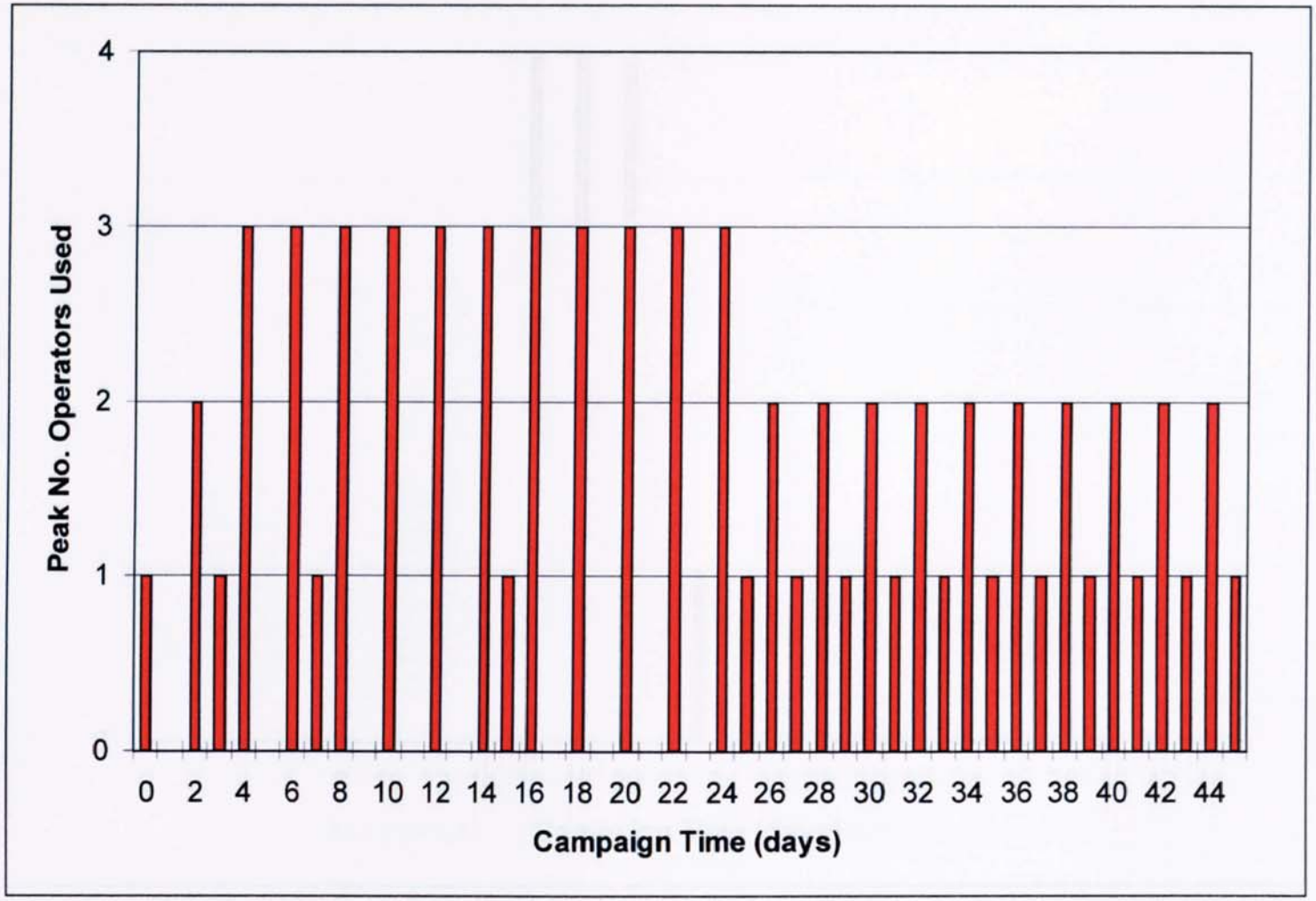

Figure 5.11 Trend chart showing the peak daily operator requirement profile for the {1S2, 2P3} operating schedule with an operator pool size of 3.

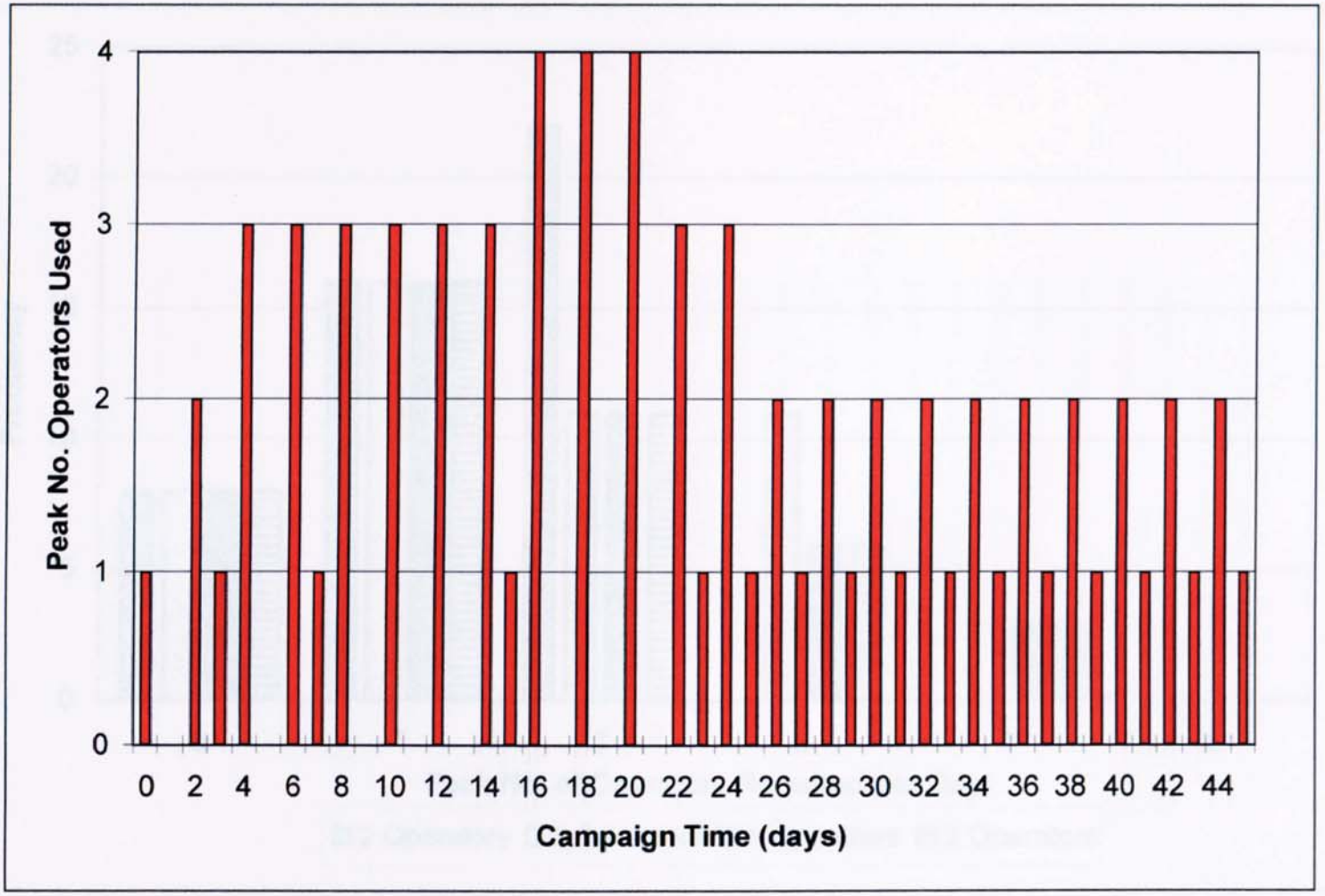

Figure 5.12 Trend chart showing the peak daily operator requirement profile for the {1S2, 2P3} operating schedule with an operator pool size of 5.

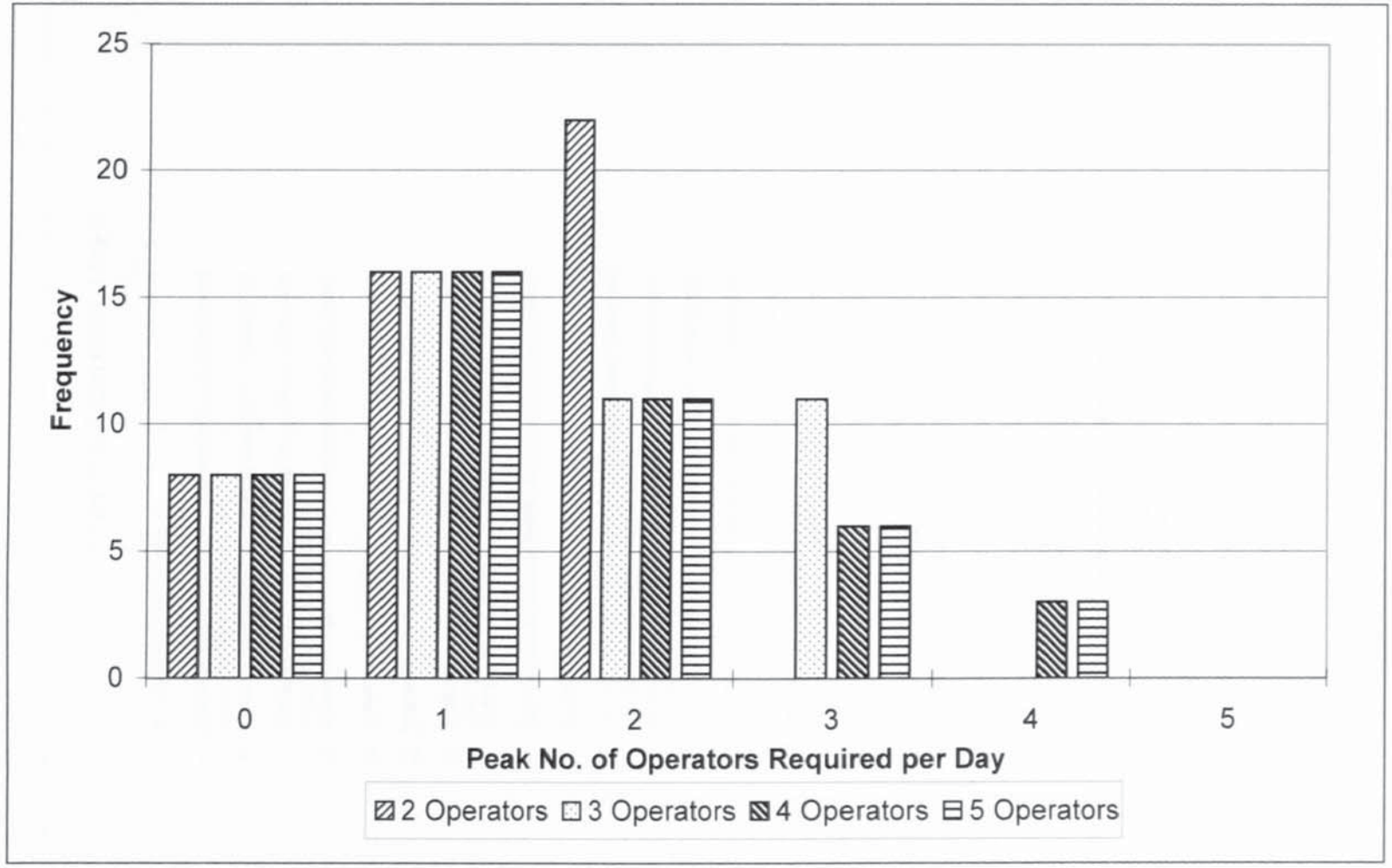

Figure 5.13 Frequency distribution profile showing the relative daily peak allocation of the available operator pool for a production campaign employing the {1S2, 2P3} schedule.

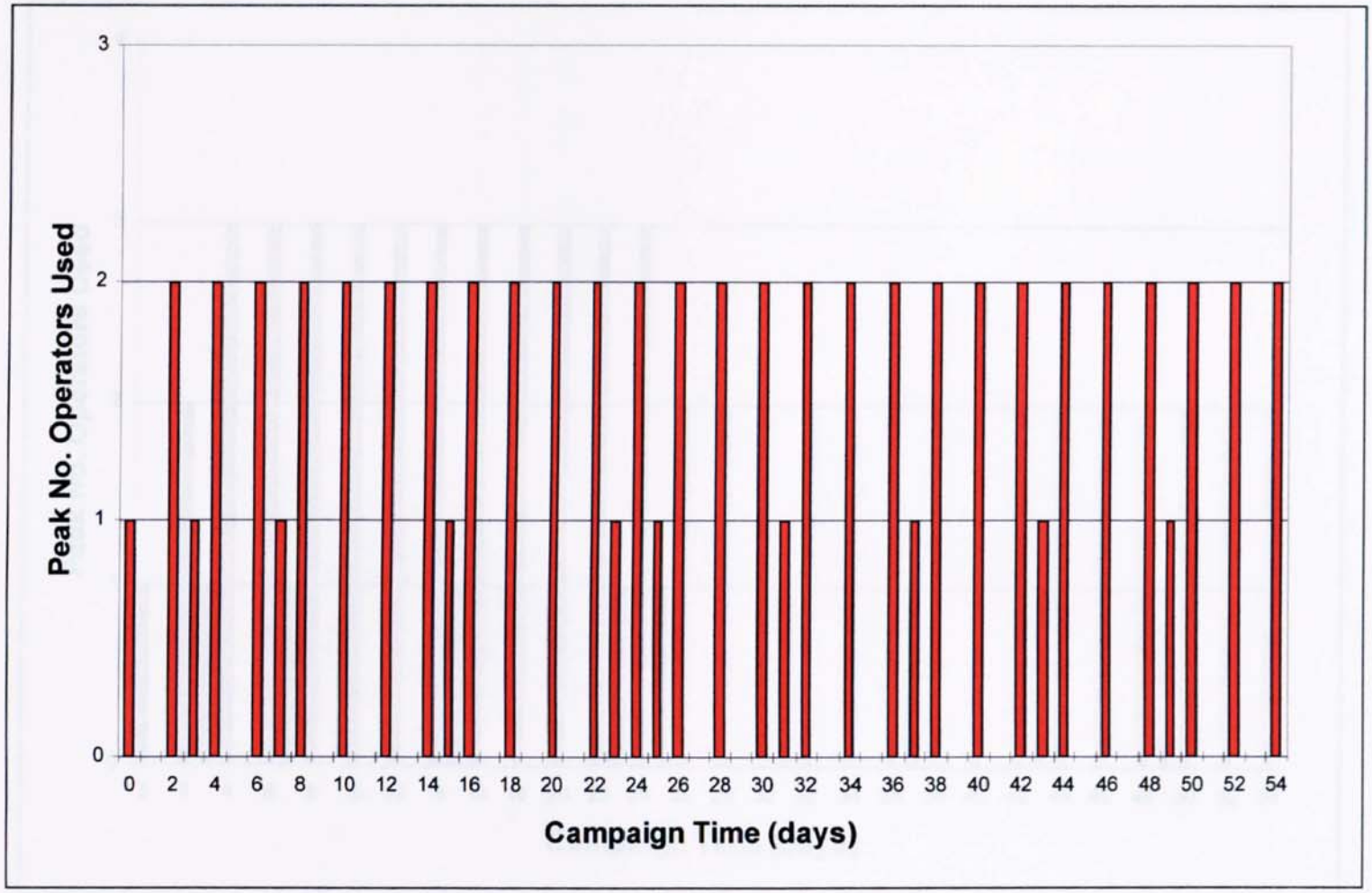

Figure 5.14 Trend chart showing the peak daily operator requirement profile for the {1S2, 2P4} operating schedule with an operator pool size of 2.

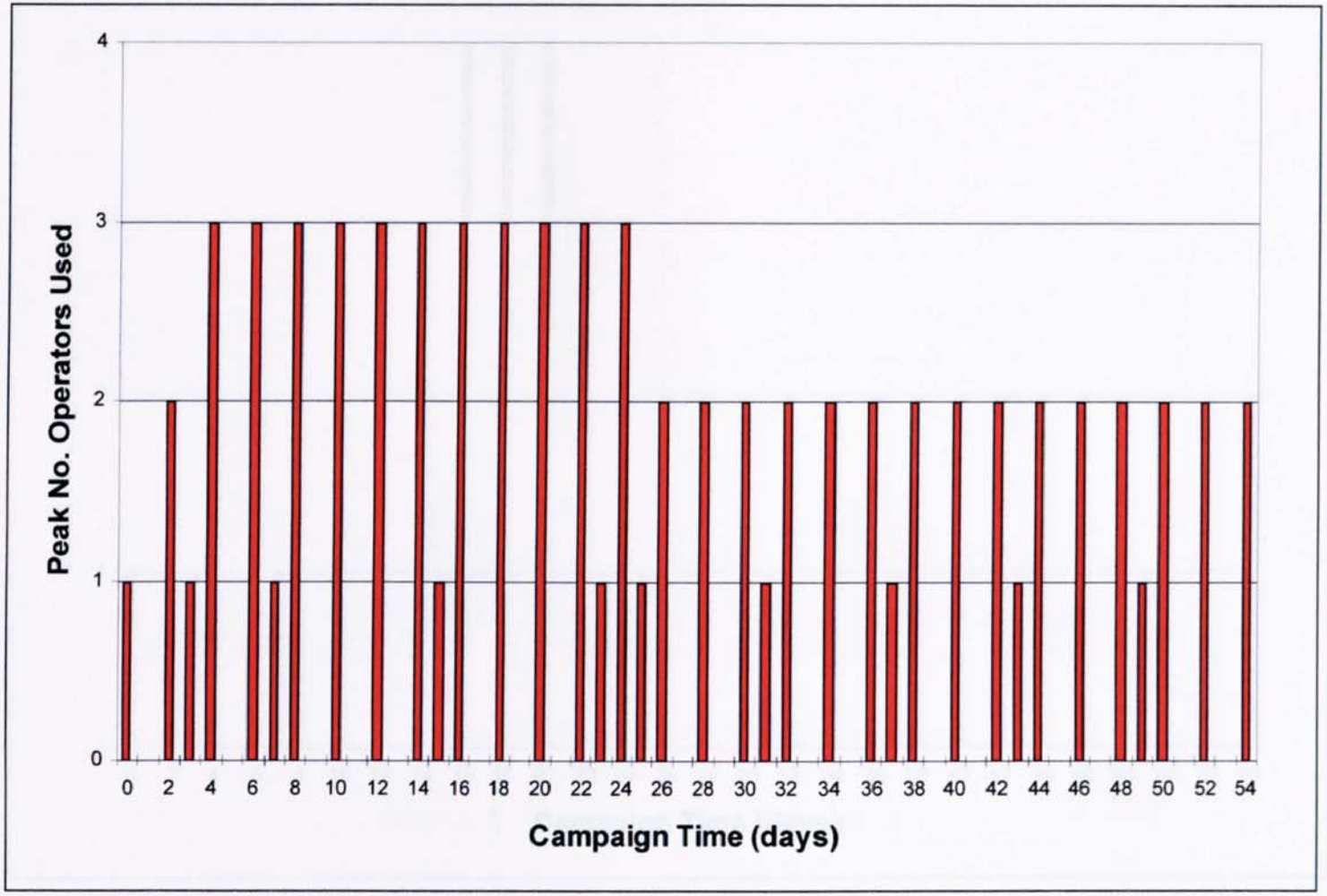

Figure 5.15 Trend chart showing the peak daily operator requirement profile for the {1S2, 2P4} operating schedule with an operator pool size of 3.

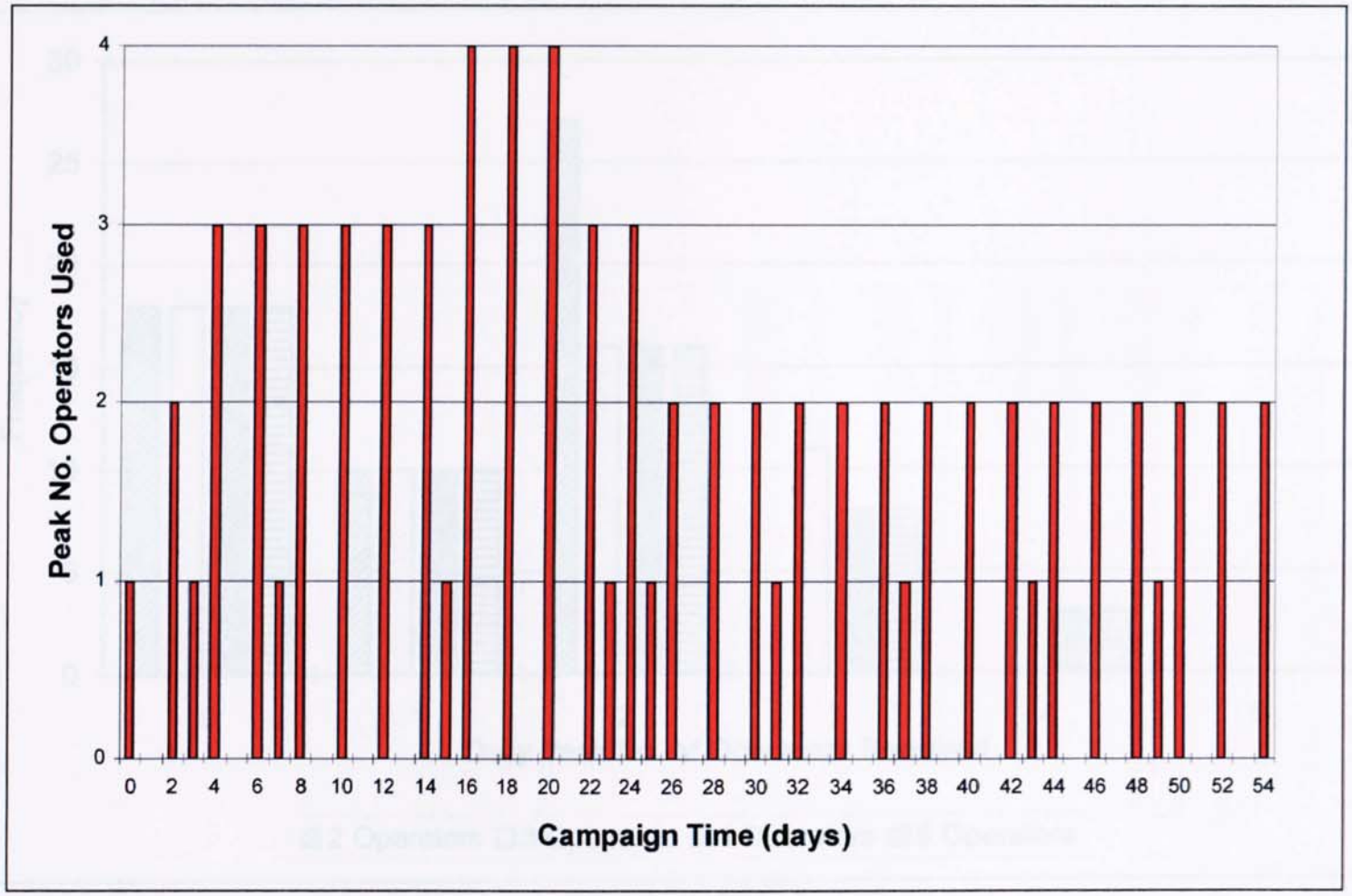

Figure 5.16 Trend chart showing the peak daily operator requirement profile for the {1S2, 2P4} operating schedule with an operator pool size of 5.

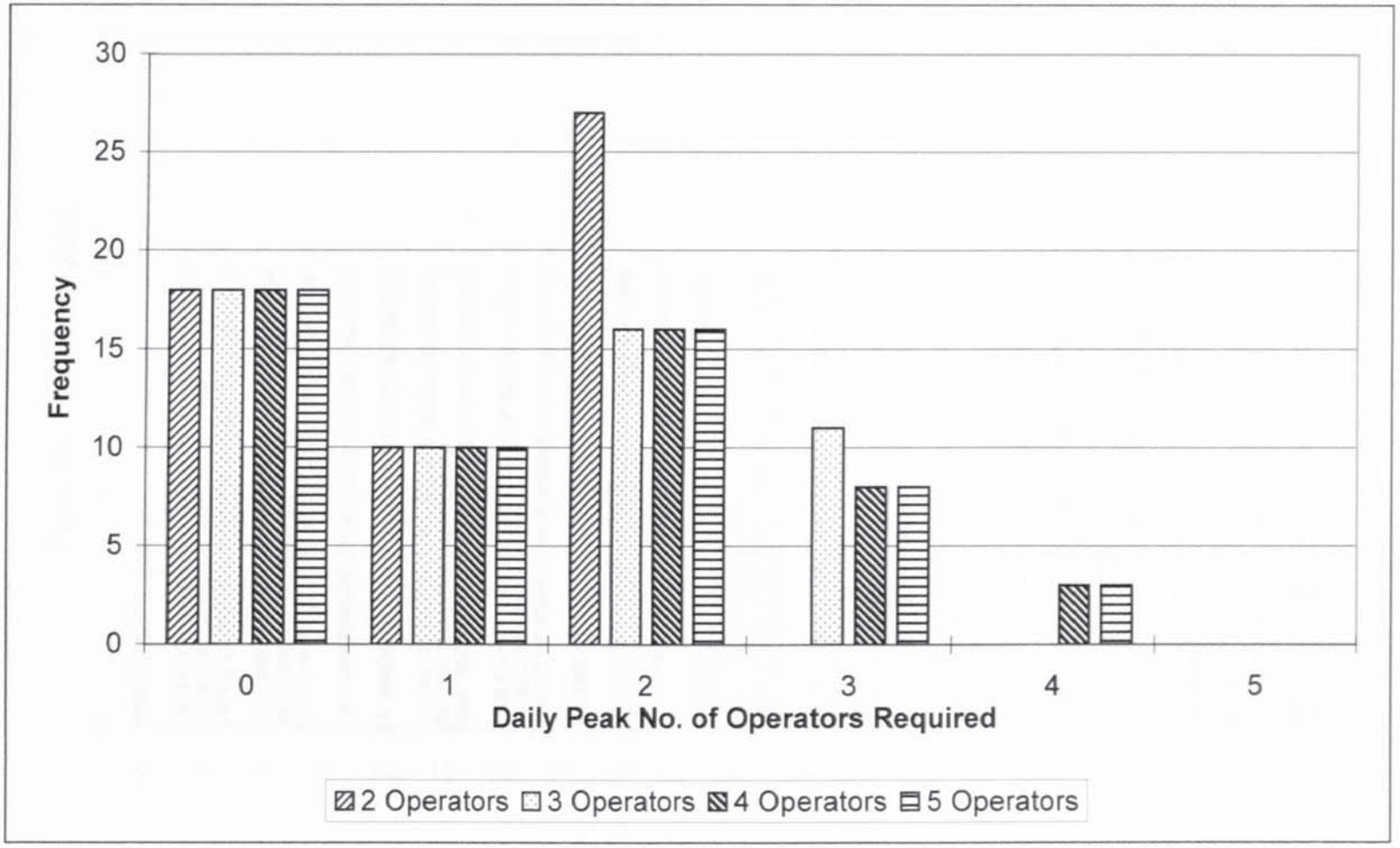

Figure 5.17 Frequency distribution profile showing the relative daily peak allocation of the available operator pool for a production campaign employing the {1S2, 2P4} schedule.

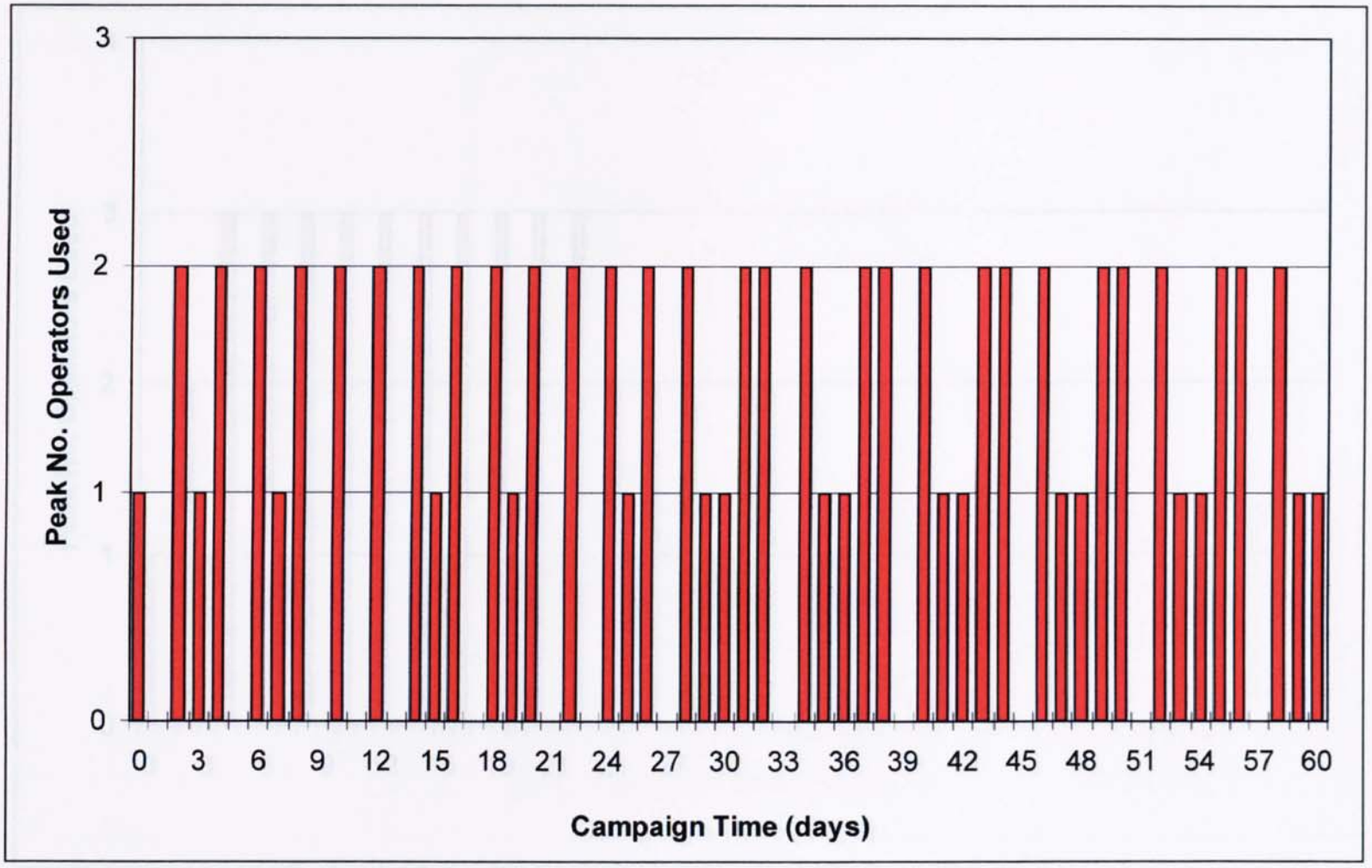

Figure 5.18 Trend chart showing the peak daily operator requirement profile for the {1S3, 2P3} operating schedule with an operator pool size of 2.

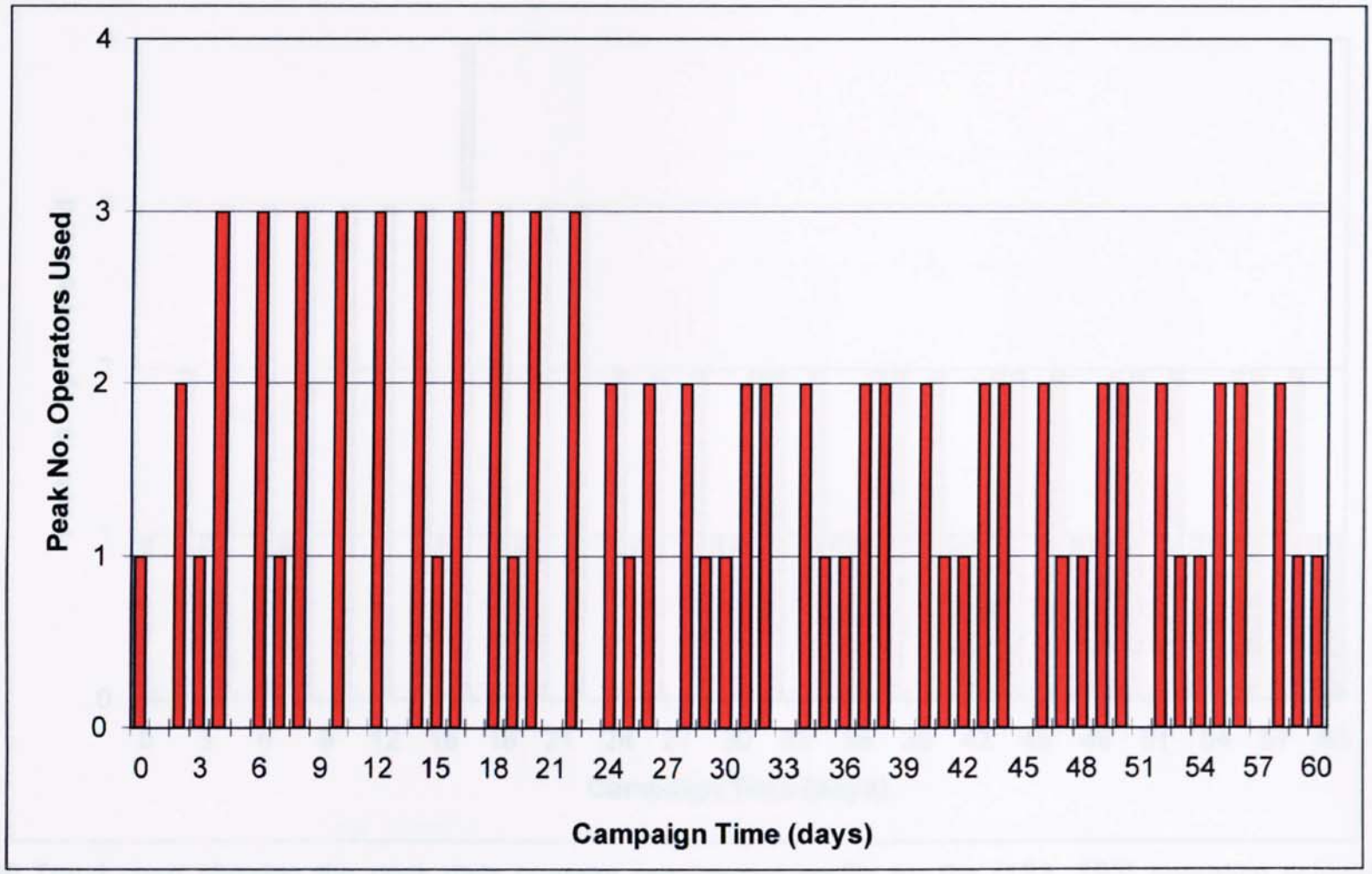

Figure 5.19 Trend chart showing the peak daily operator requirement profile for the {1S3, 2P3} operating schedule with an operator pool size of 3.

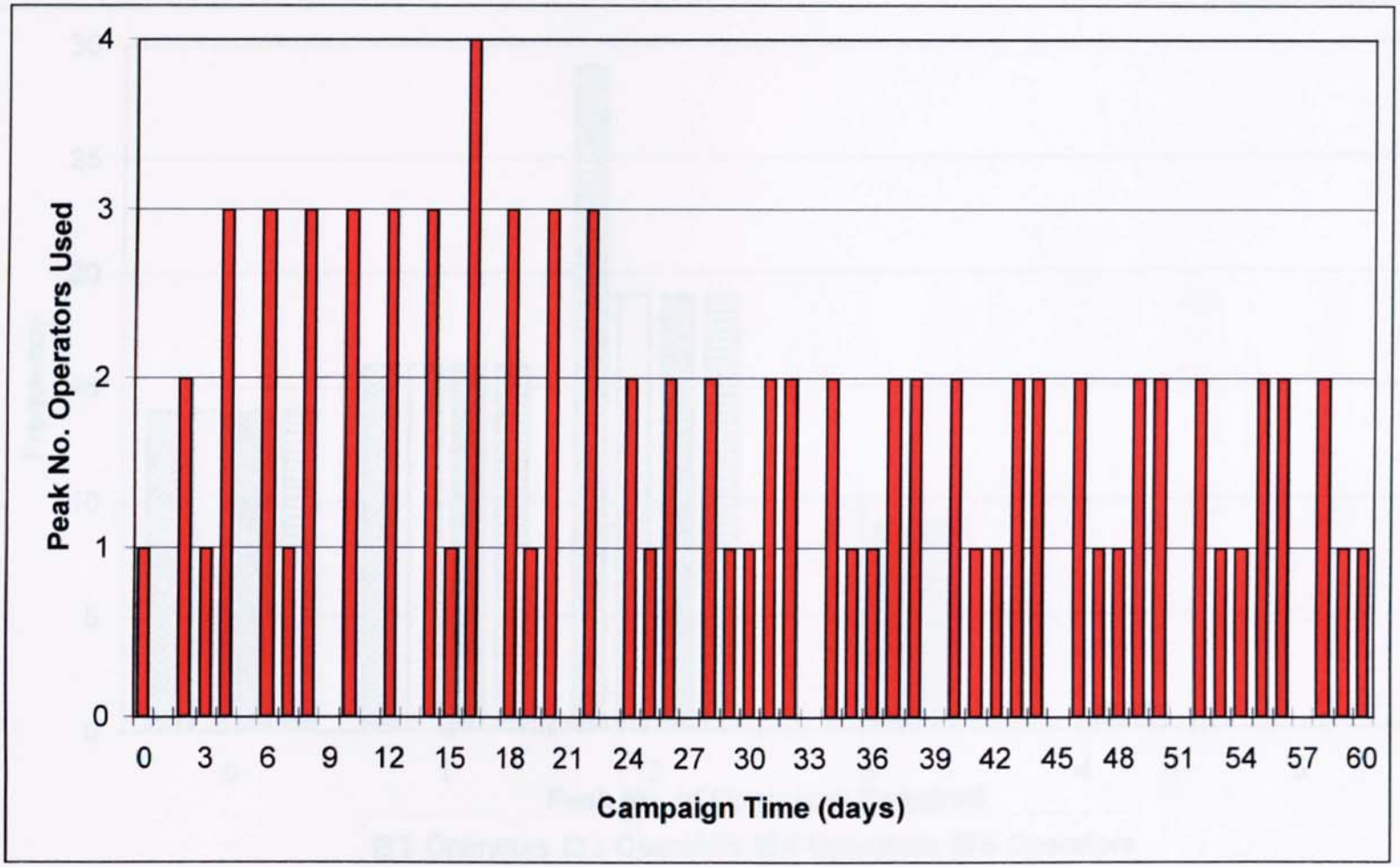

Figure 5.20 Trend chart showing the peak daily operator requirement profile for the {1S3, 2P3} operating schedule with an operator pool size of 5.

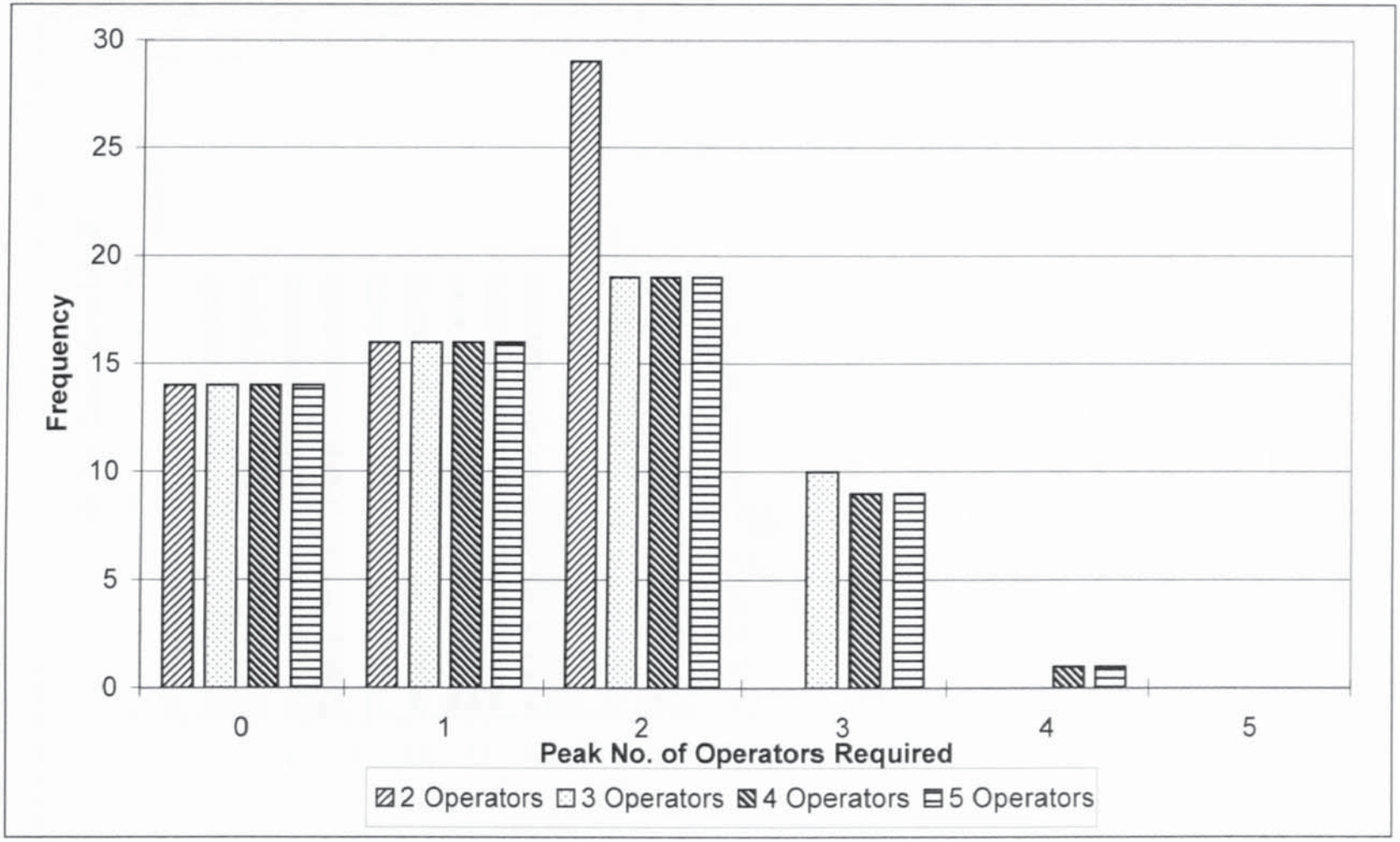

Figure 5.21 Frequency distribution profile showing the relative daily peak allocation of the available operator pool for a production campaign employing the {1S3, 2P3} schedule.

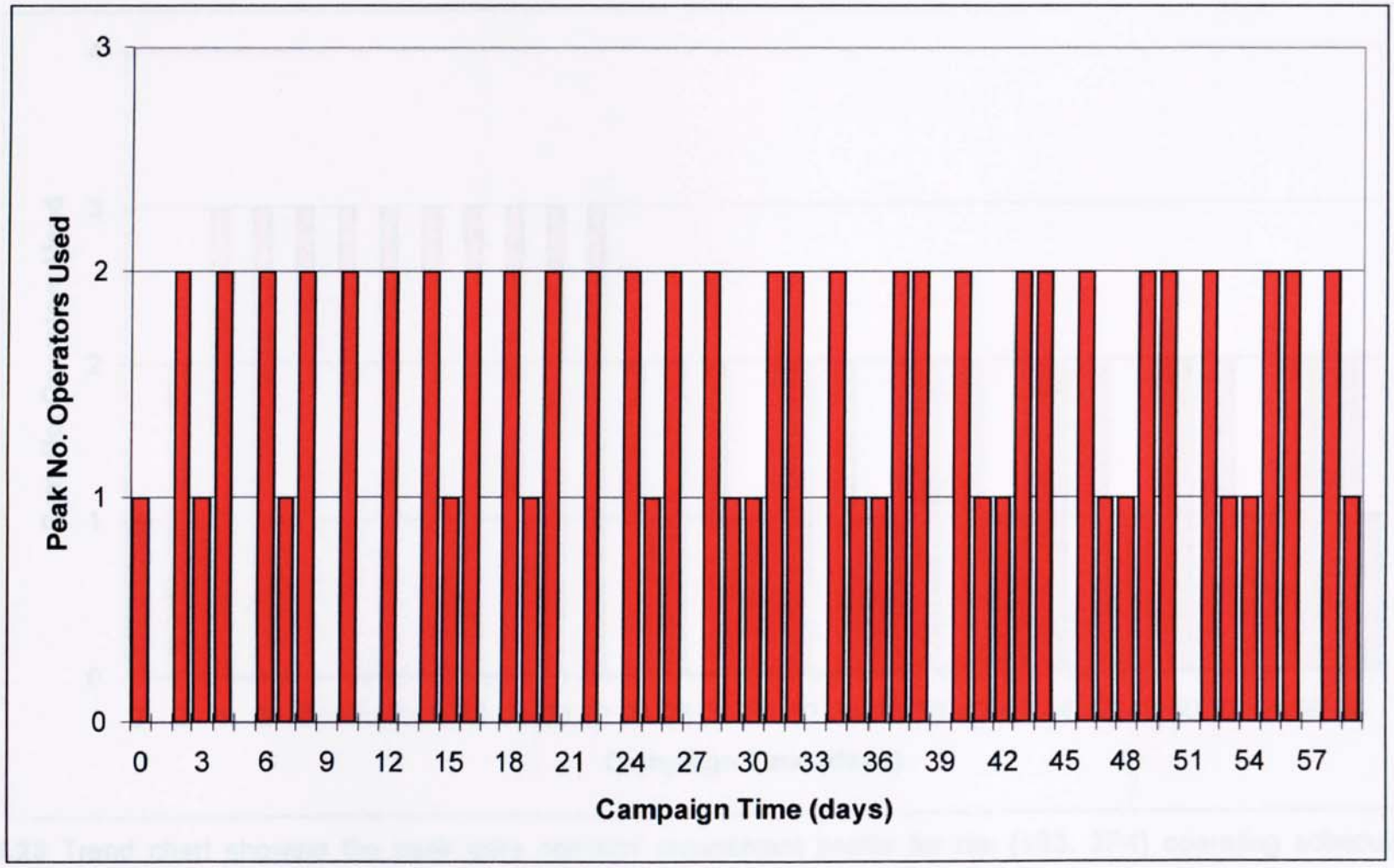

Figure 5.22 Trend chart showing the peak daily operator requirement profile for the {1S3, 2P4} operating schedule with an operator pool size of 2.

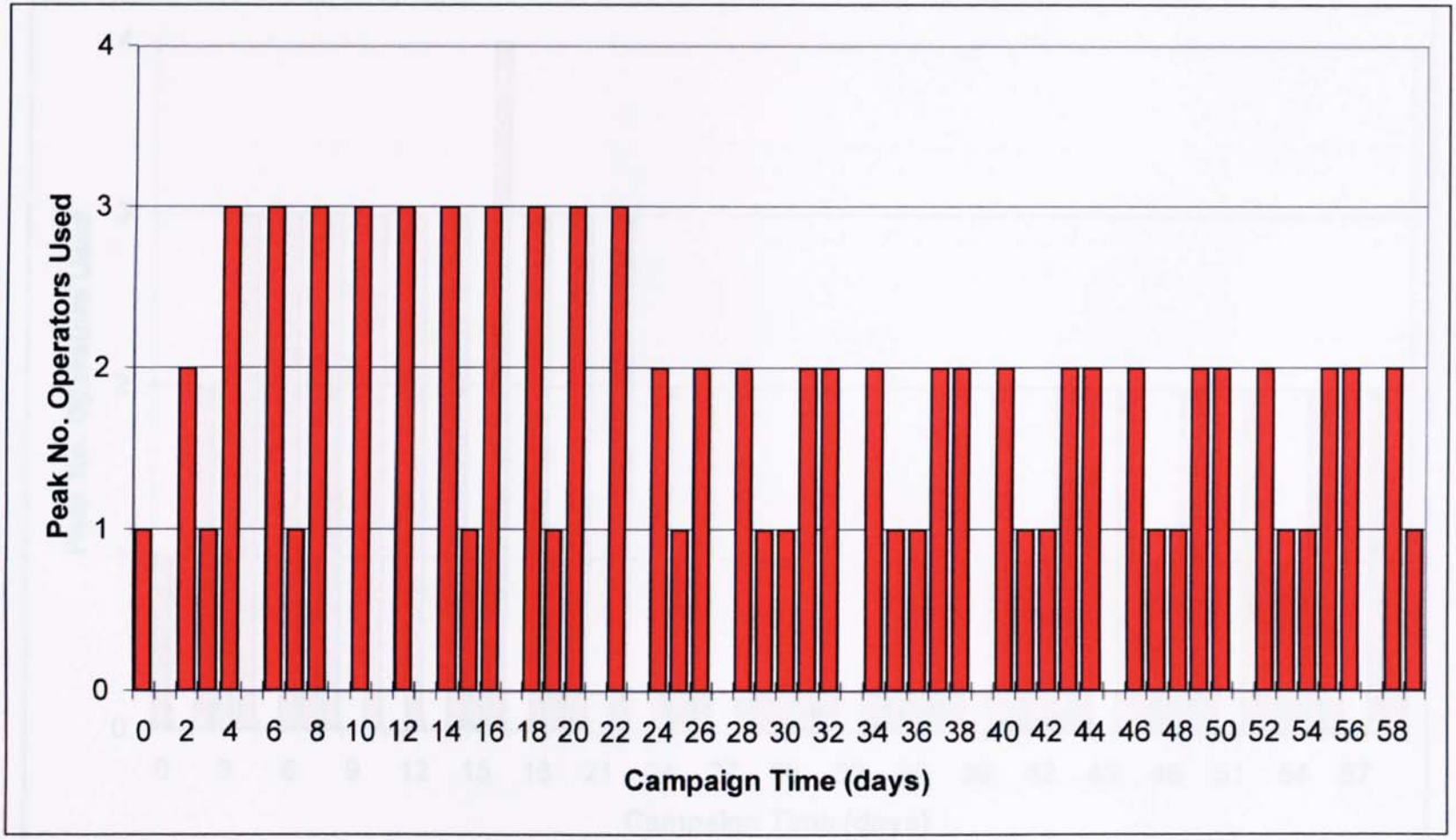

Figure 5.23 Trend chart showing the peak daily operator requirement profile for the {1S3, 2P4} operating schedule with an operator pool size of 3.

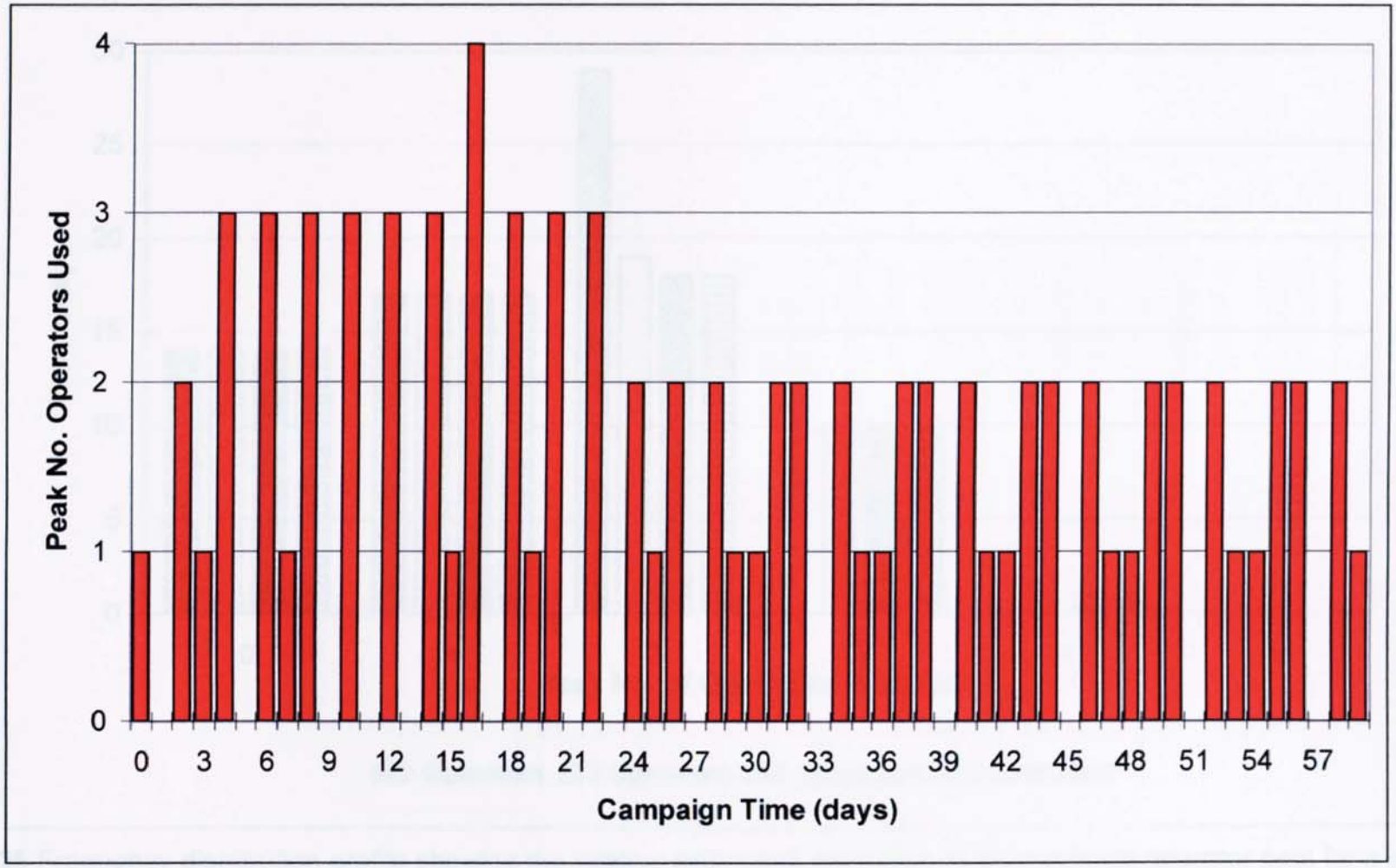

Figure 5.24 Trend chart showing the peak daily operator requirement profile for the {1S3, 2P4} operating schedule with an operator pool size of 5.

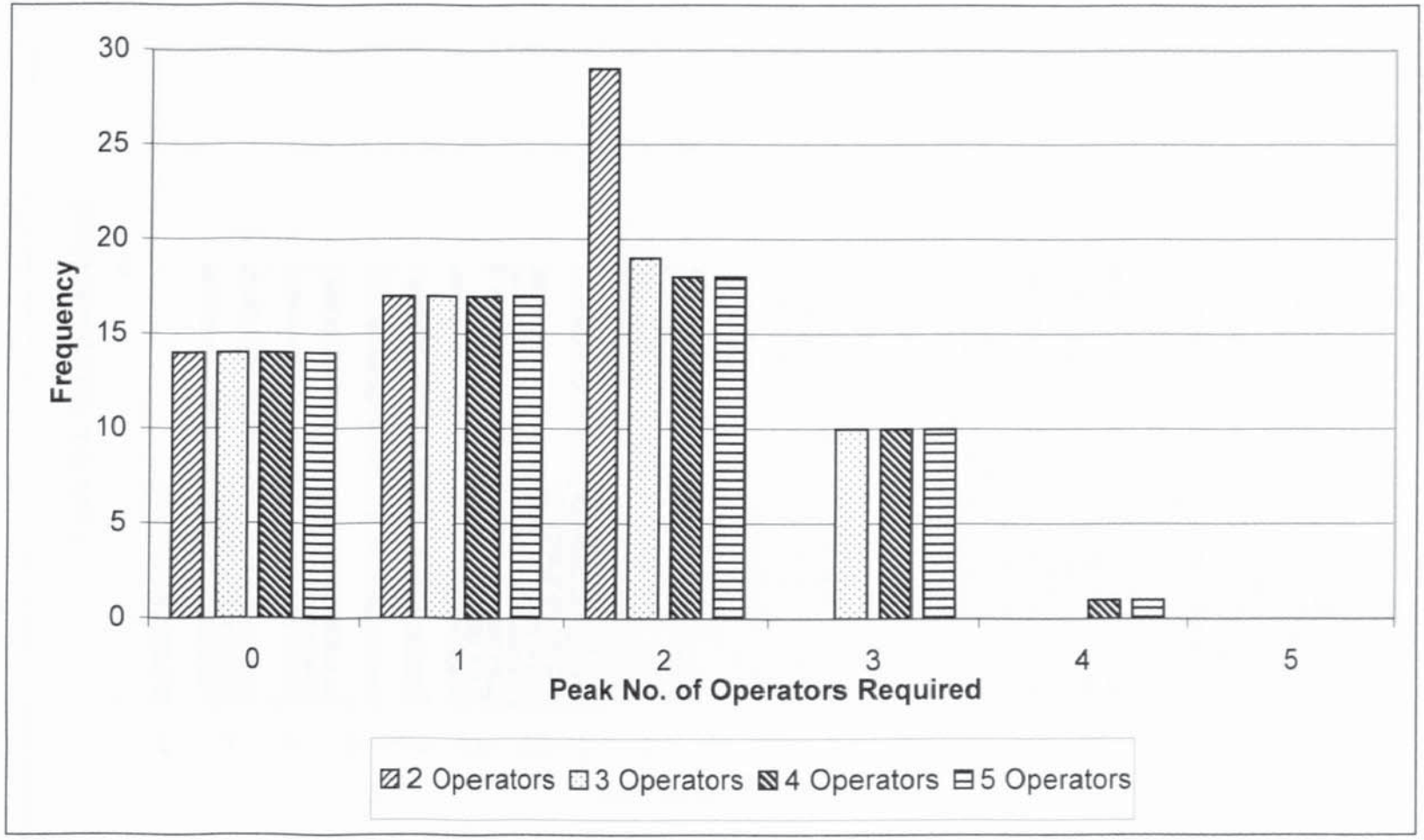

Figure 5.25 Frequency distribution profile showing the relative daily peak allocation of the available operator pool for a production campaign employing the {1S3, 2P4} schedule.

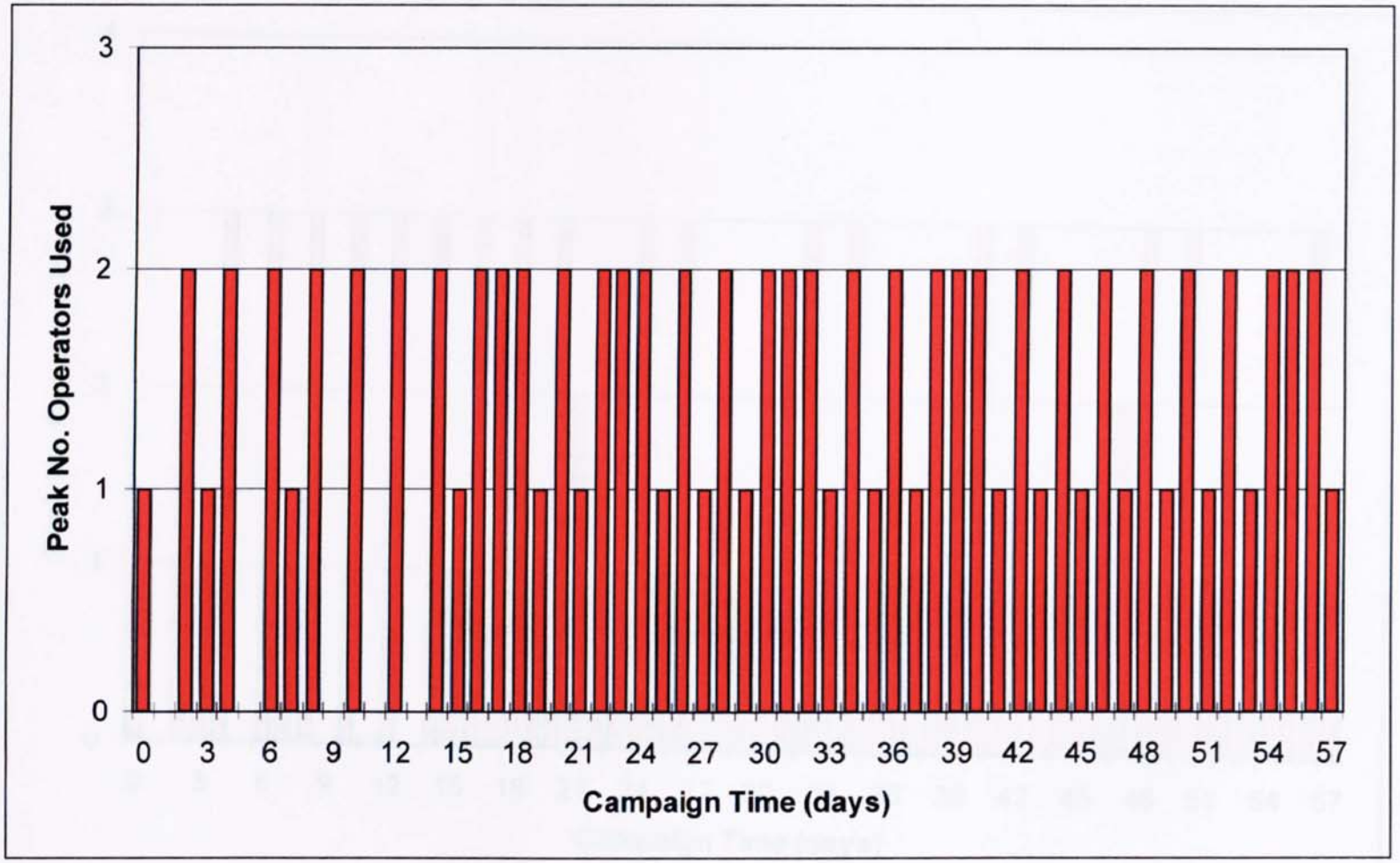

Figure 5.26 Trend chart showing the peak daily operator requirement profile for the {1S2, 2P7} operating schedule with an operator pool size of 2.

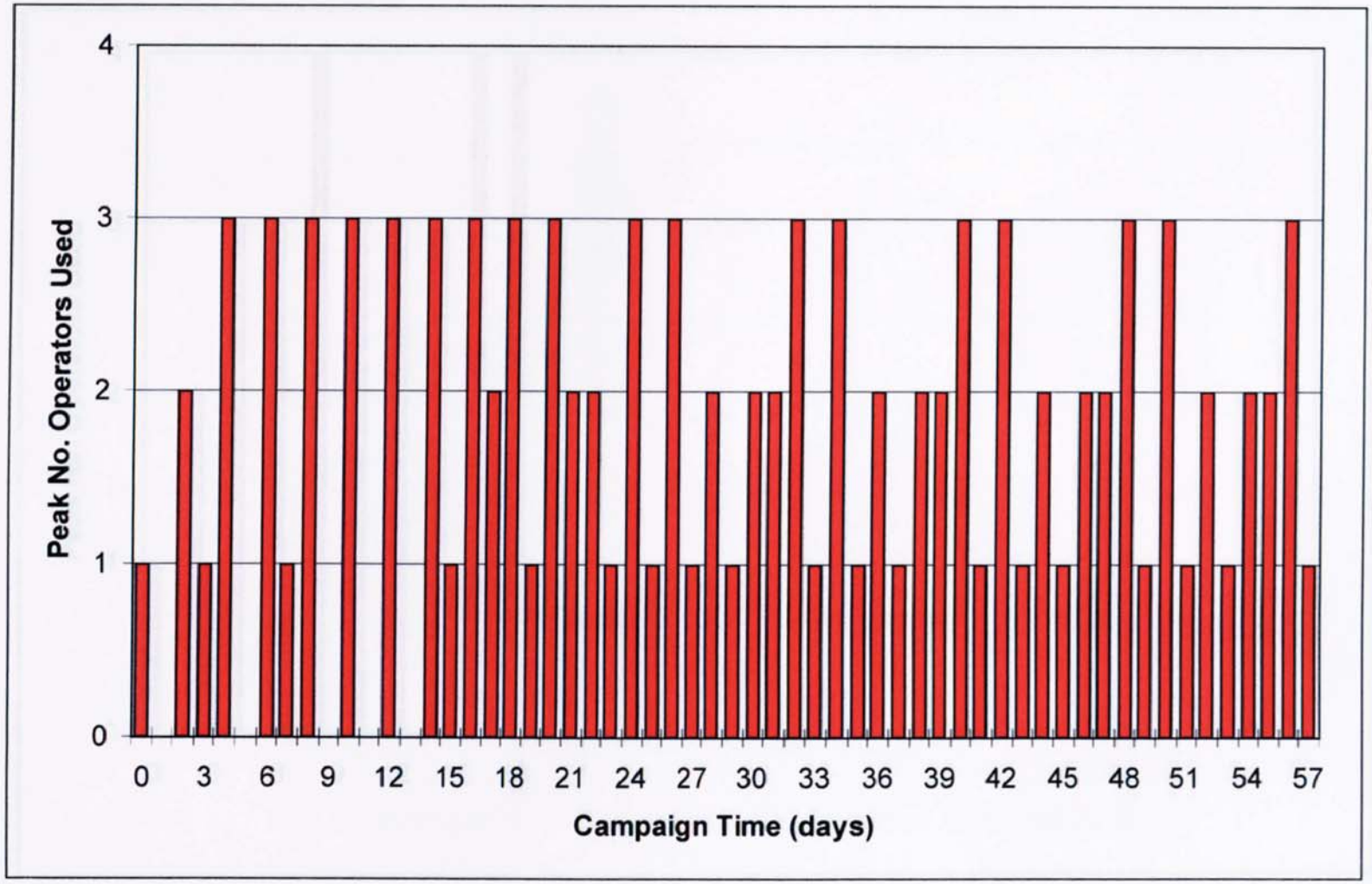

Figure 5.27 Trend chart showing the peak daily operator requirement profile for the {1S2, 2P7} operating schedule with an operator pool size of 3.

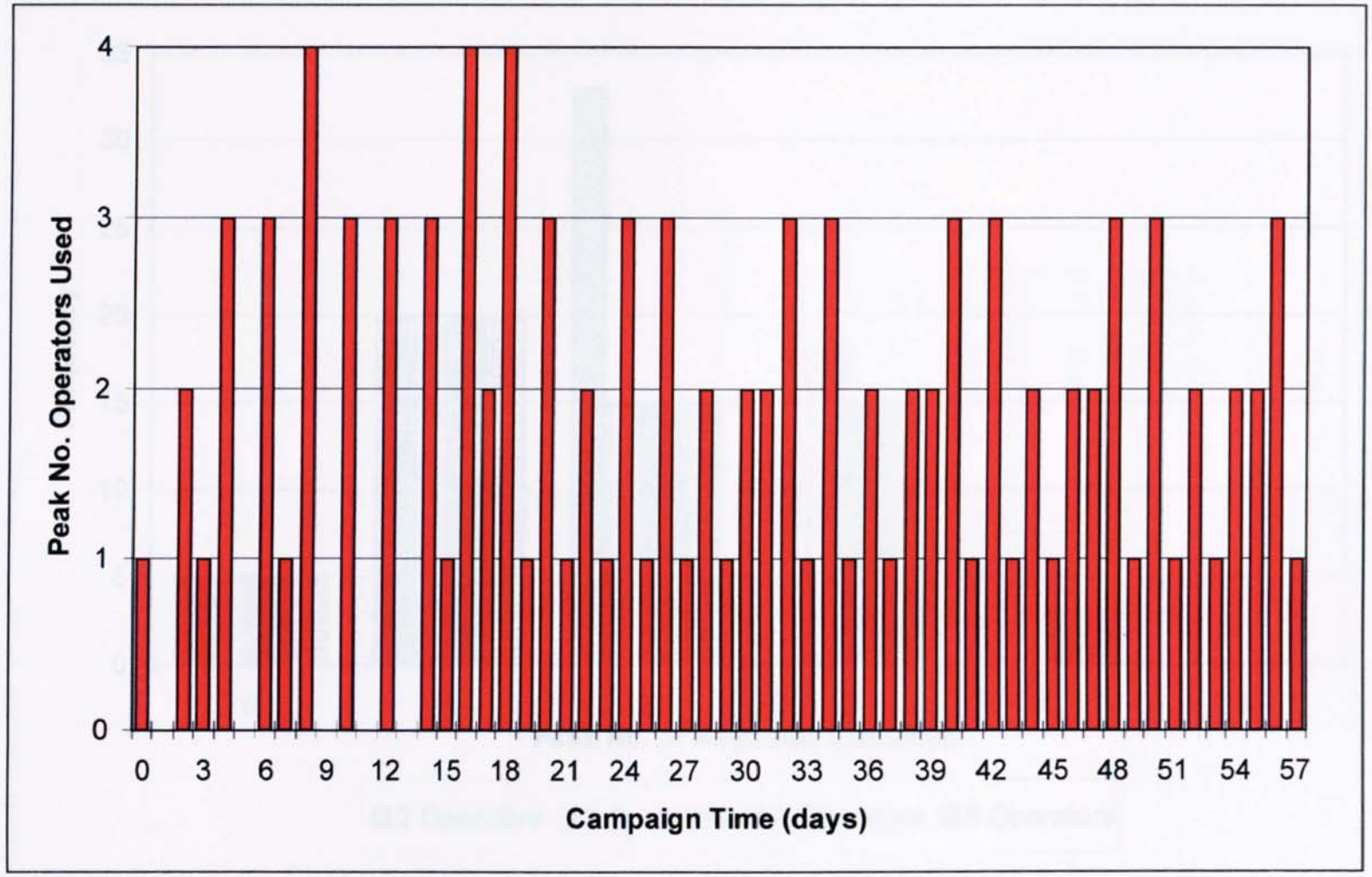

Figure 5.28 Trend chart showing the peak daily operator requirement profile for the {1S2, 2P7} operating schedule with an operator pool size of 5.

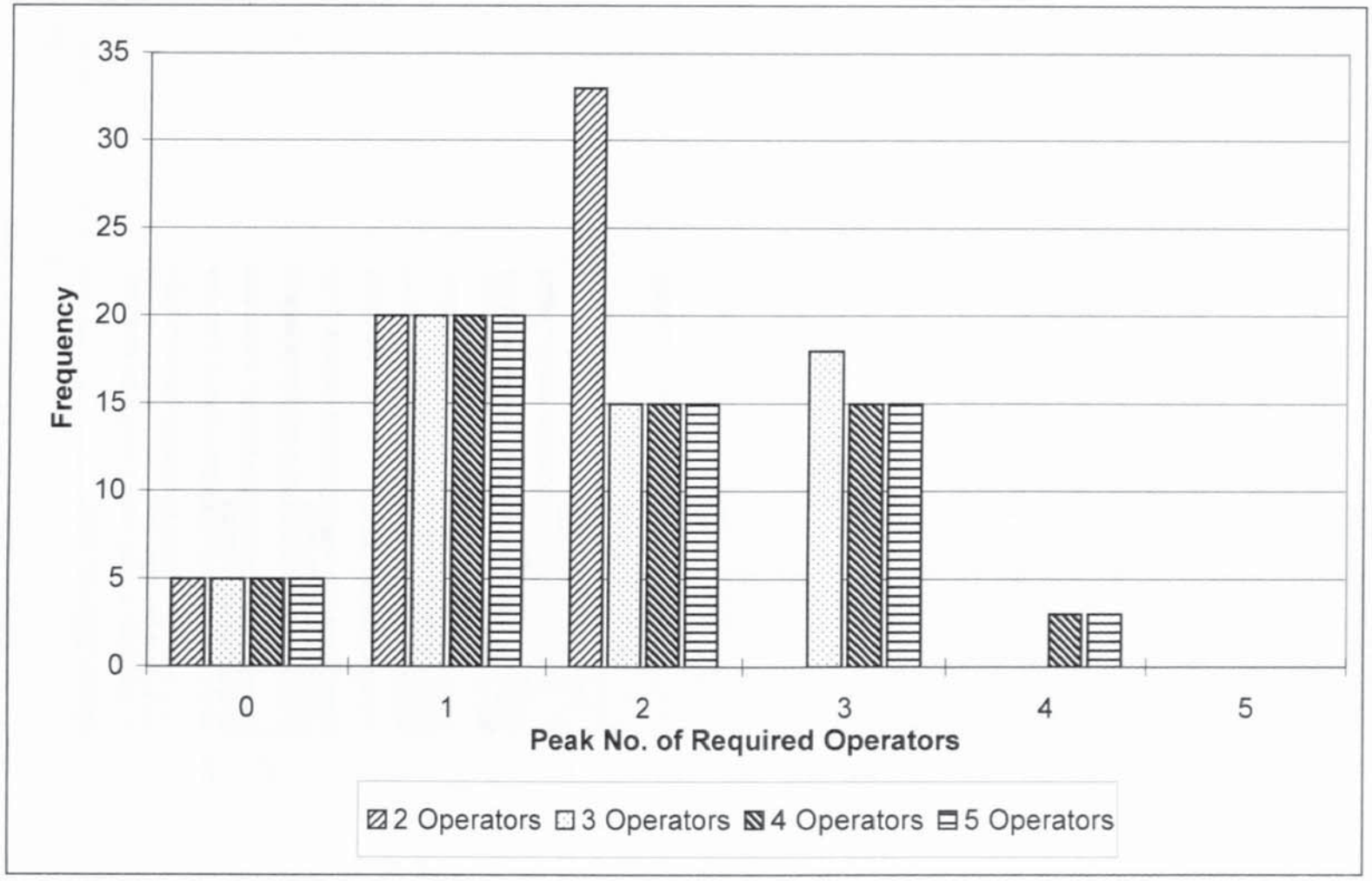

Figure 5.29 Frequency distribution profile showing the relative daily peak allocation of the available operator pool for a production campaign employing the {1S2, 2P7} schedule.

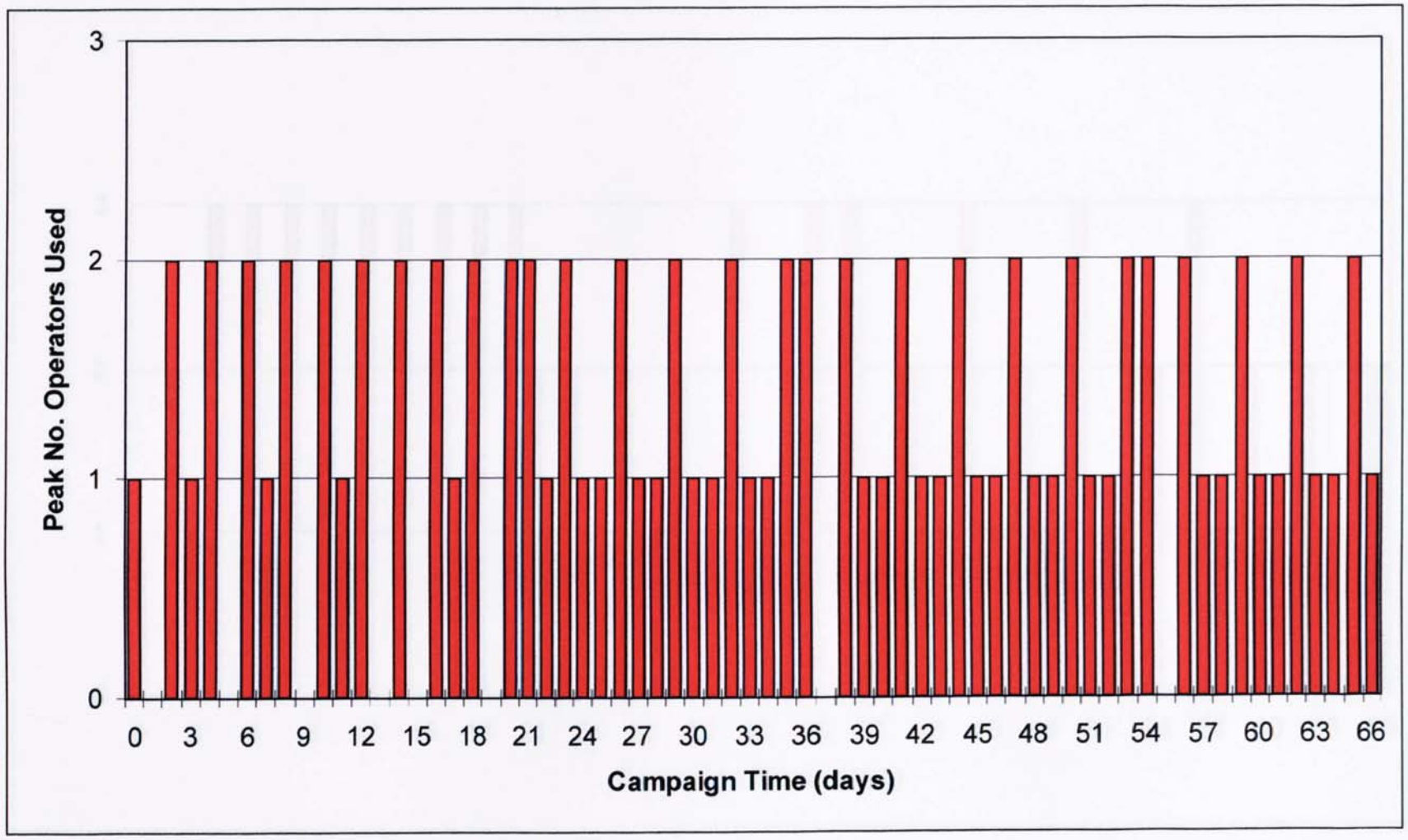

Figure 5.30 Trend chart showing the peak daily operator requirement profile for the {1S3, 2P7} operating schedule with an operator pool size of 2.

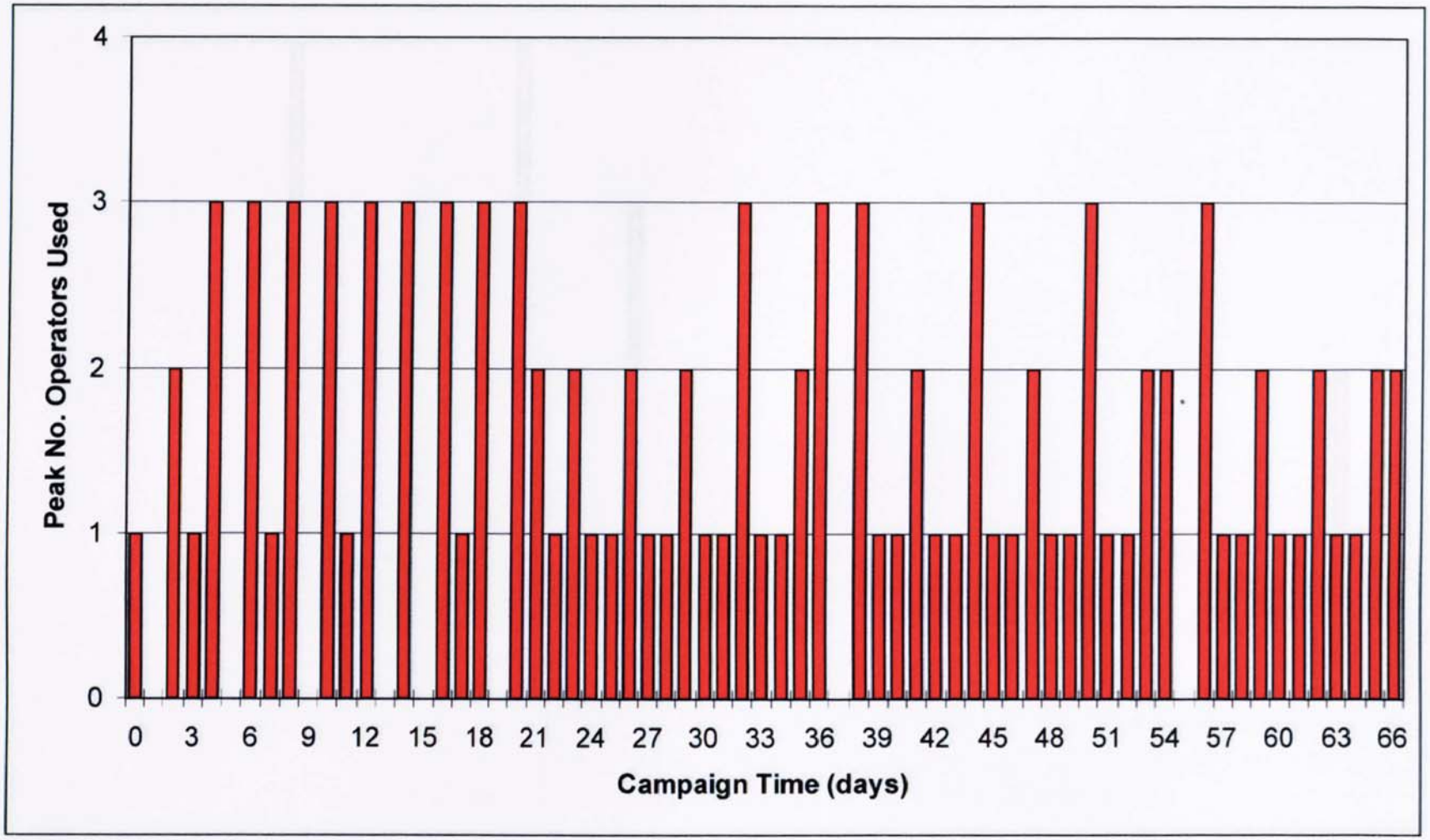

Figure 5.31 Trend chart showing the peak daily operator requirement profile for the {1S3, 2P7} operating schedule with an operator pool size of 3.

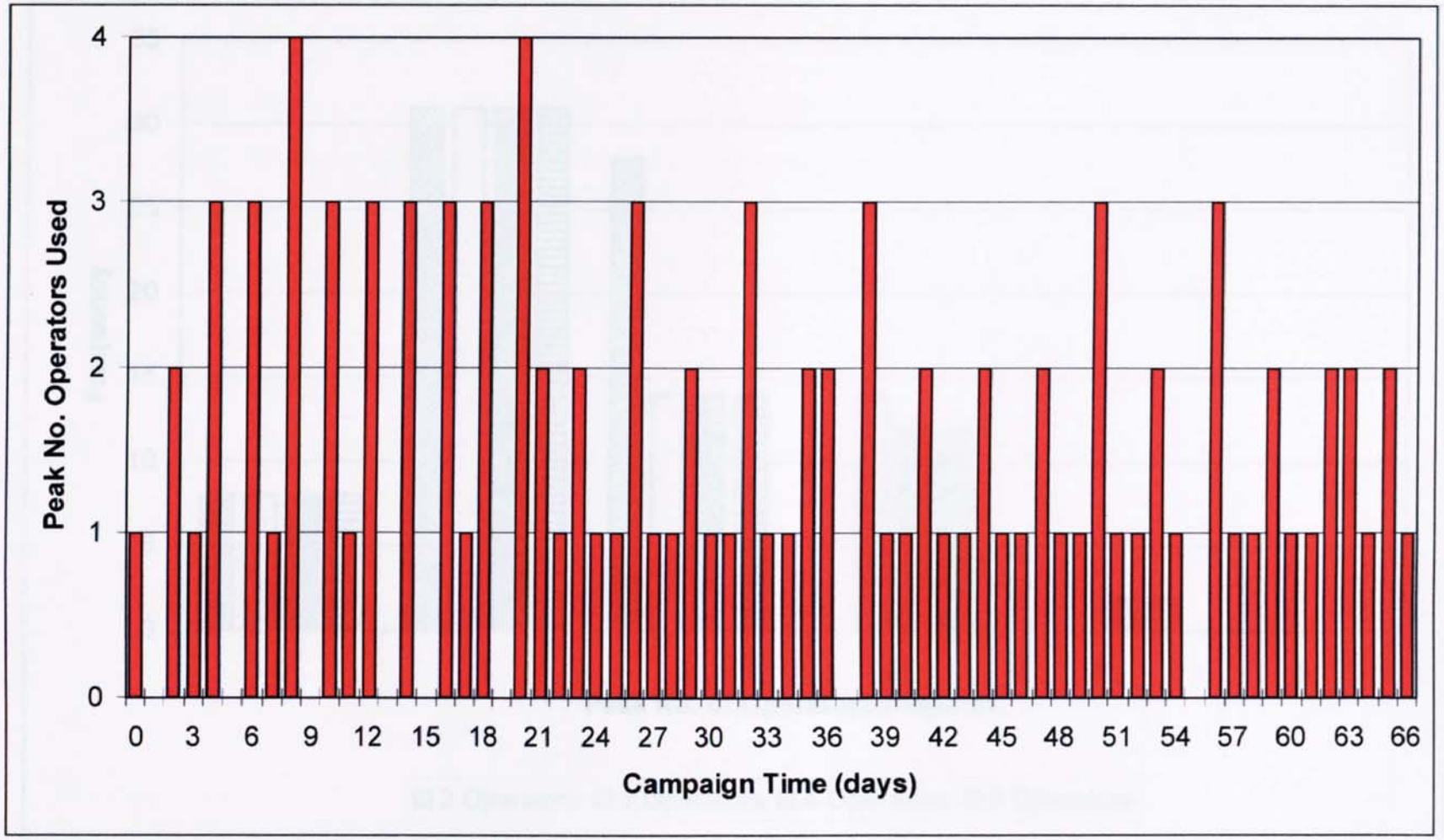

Figure 5.32 Trend chart showing the peak daily operator requirement profile for the {1S3, 2P7} operating schedule with an operator pool size of 5.

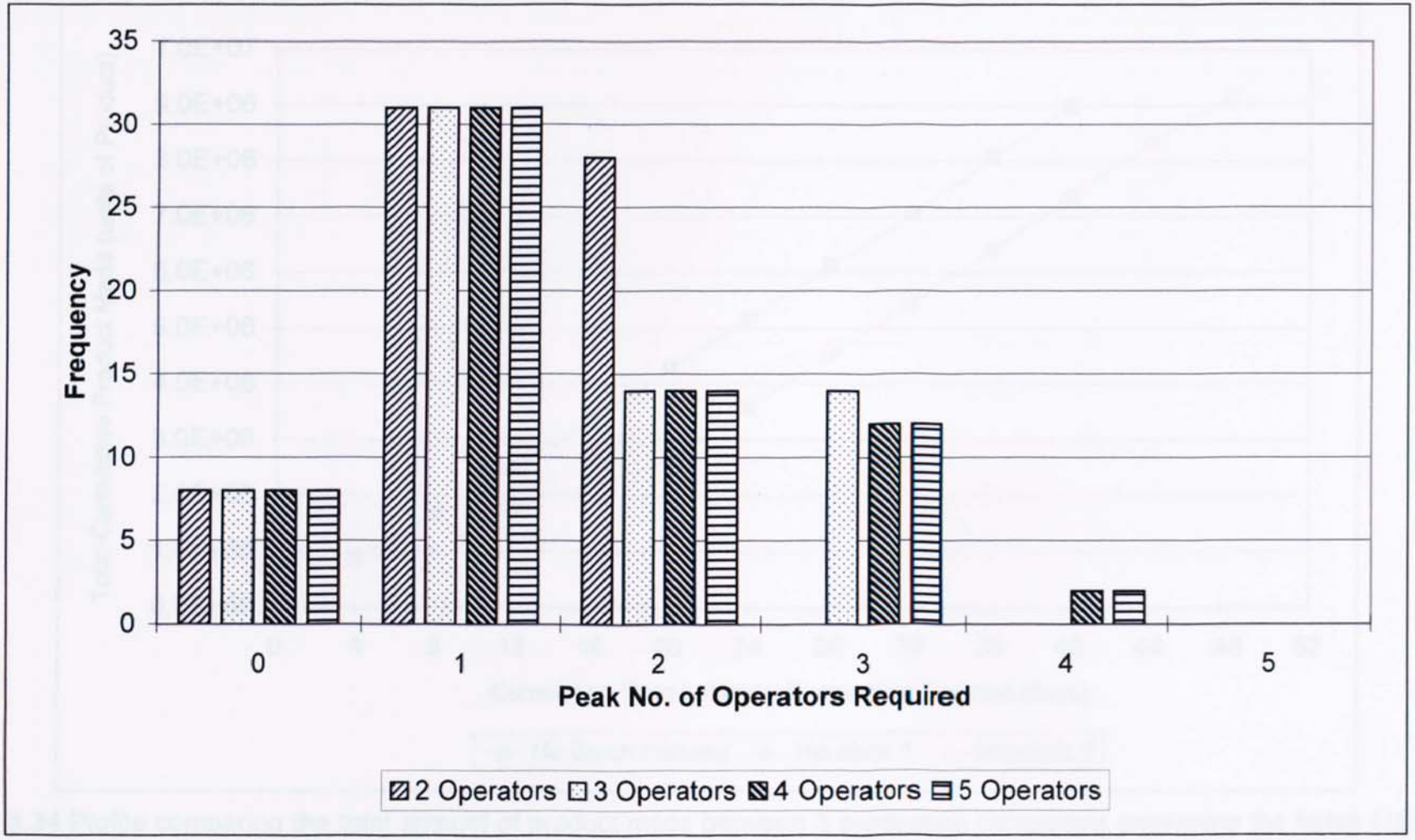

Figure 5.33. Frequency distribution profile showing the relative daily peak allocation of the available operator pool for a production campaign employing the {1S3, 2P7} schedule.

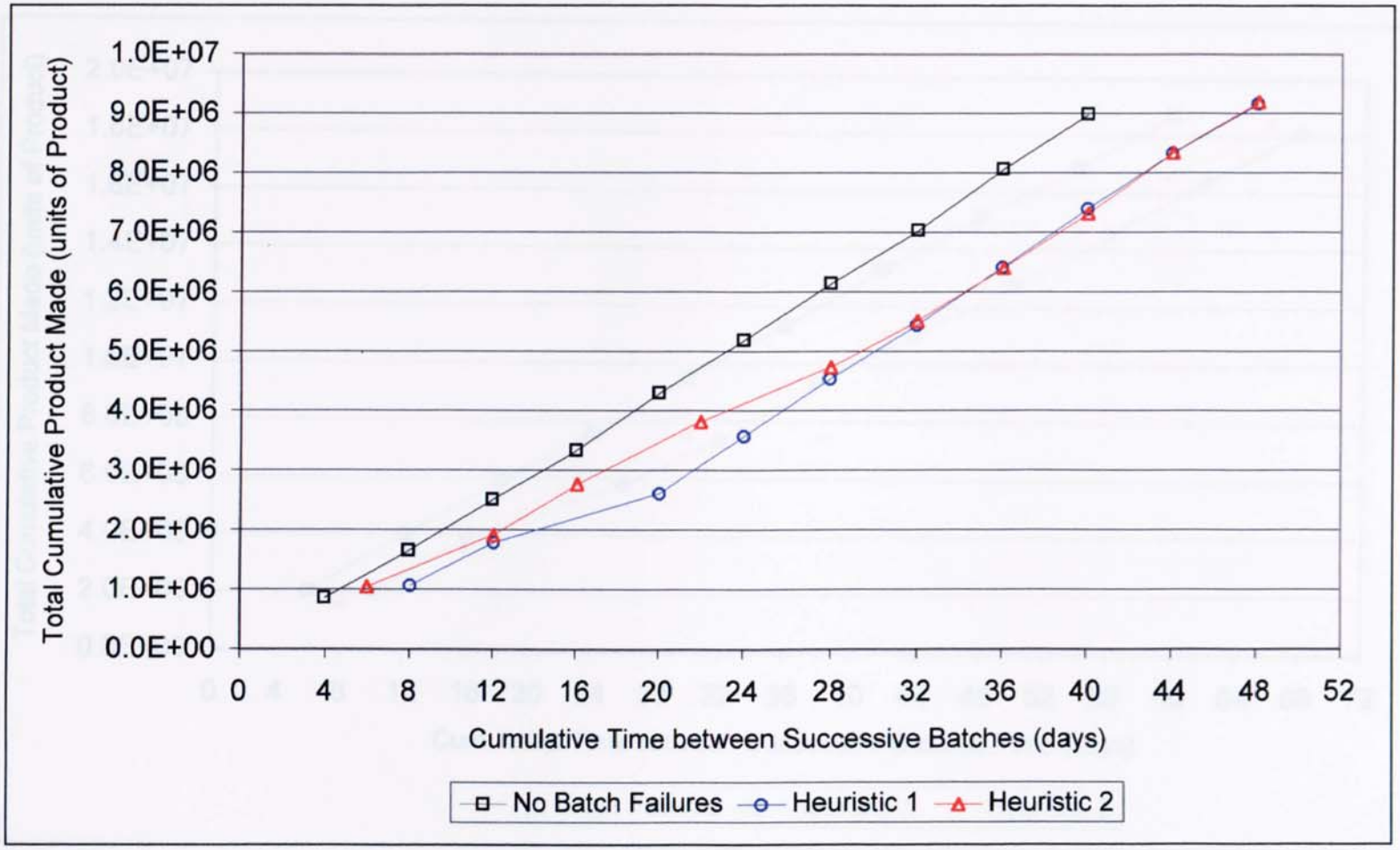

Figure 5.34 Profile comparing the total amount of product made between 3 production campaigns employing the batch {1S2 2P3} operating schedule, where 2 of the campaigns have been subject to batch failures due to contamination. For both campaigns where failures have occurred two different schedule recovery heuristics are evaluated.(Start time basis = 24 days)

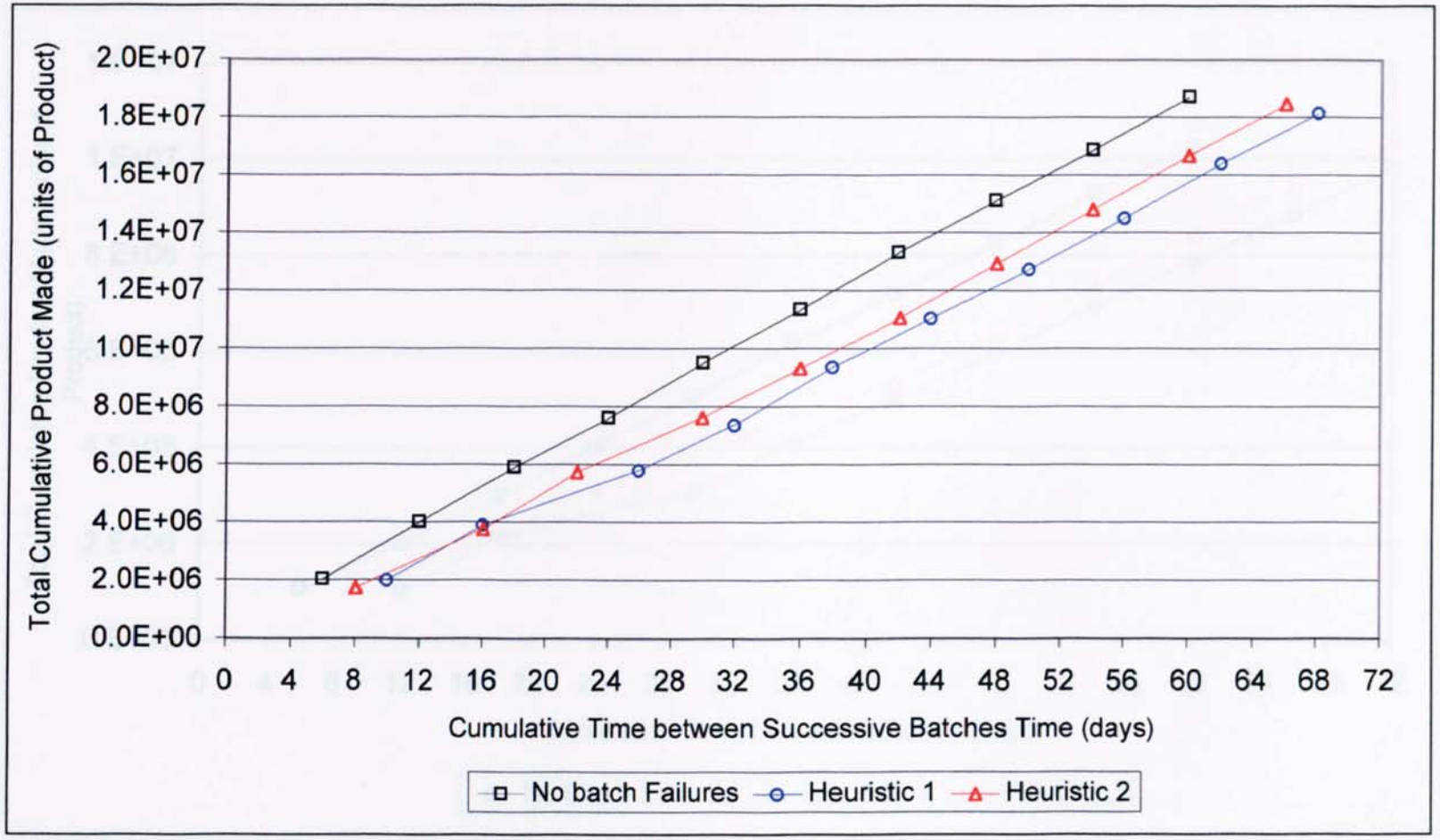

Figure 5.35 Profile comparing the total amount of product made between 3 production campaigns employing the batch {1S2 2P4} operating schedule, where 2 of the campaigns have been subject to batch failures due to contamination. For both campaigns where batch failures have occurred, two different schedule recovery heuristics are evaluated.(Start time basis = 24 days)

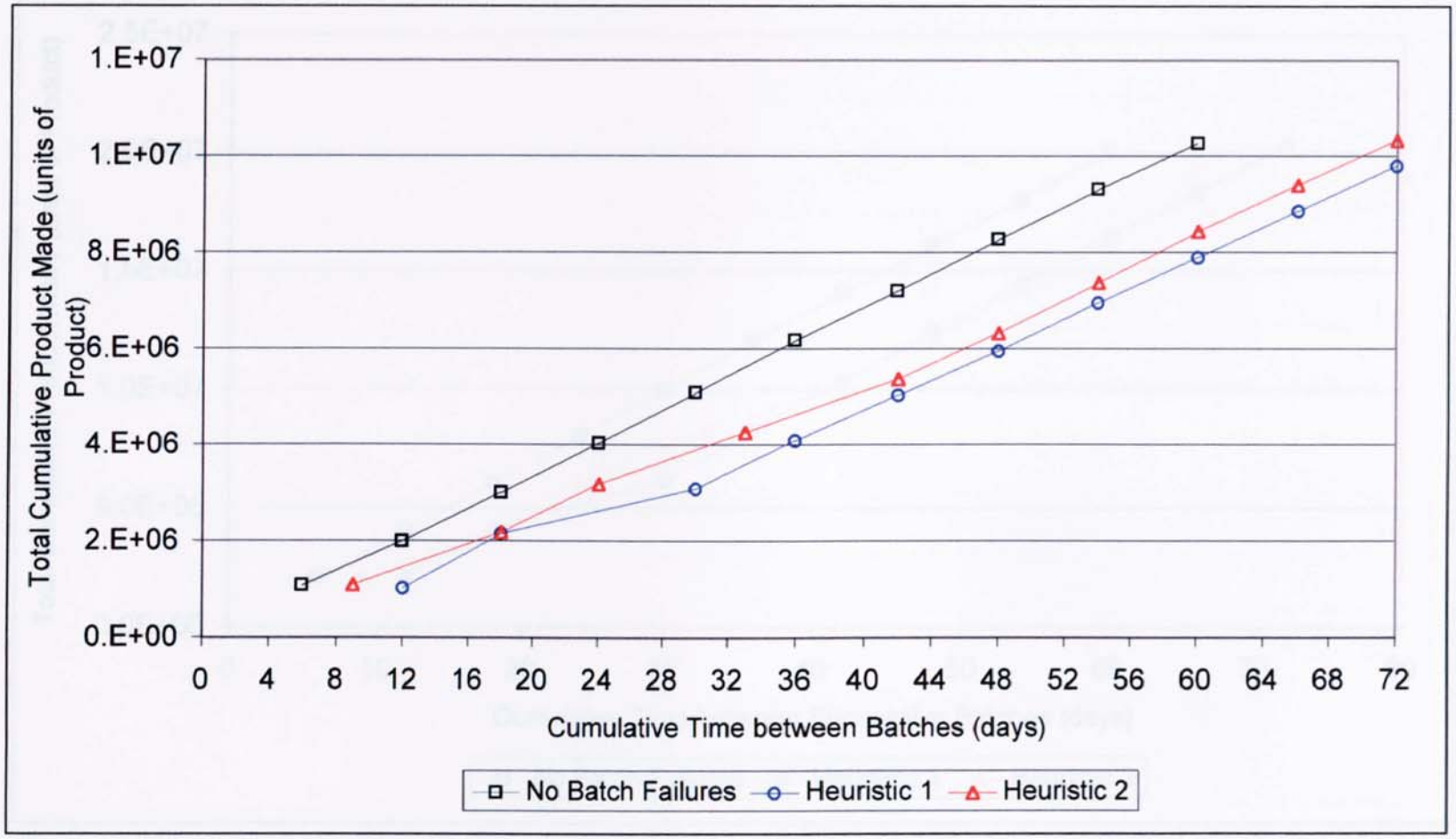

Figure 5.36 Profile comparing the total amount of product made between 3 production campaigns employing the batch {1S3 2P3} operating schedule, where 2 of the campaigns have been subject to batch failures due to contamination. For both campaigns where batch failures have occurred, two different schedule recovery heuristics are evaluated. (Start time basis =28 days).

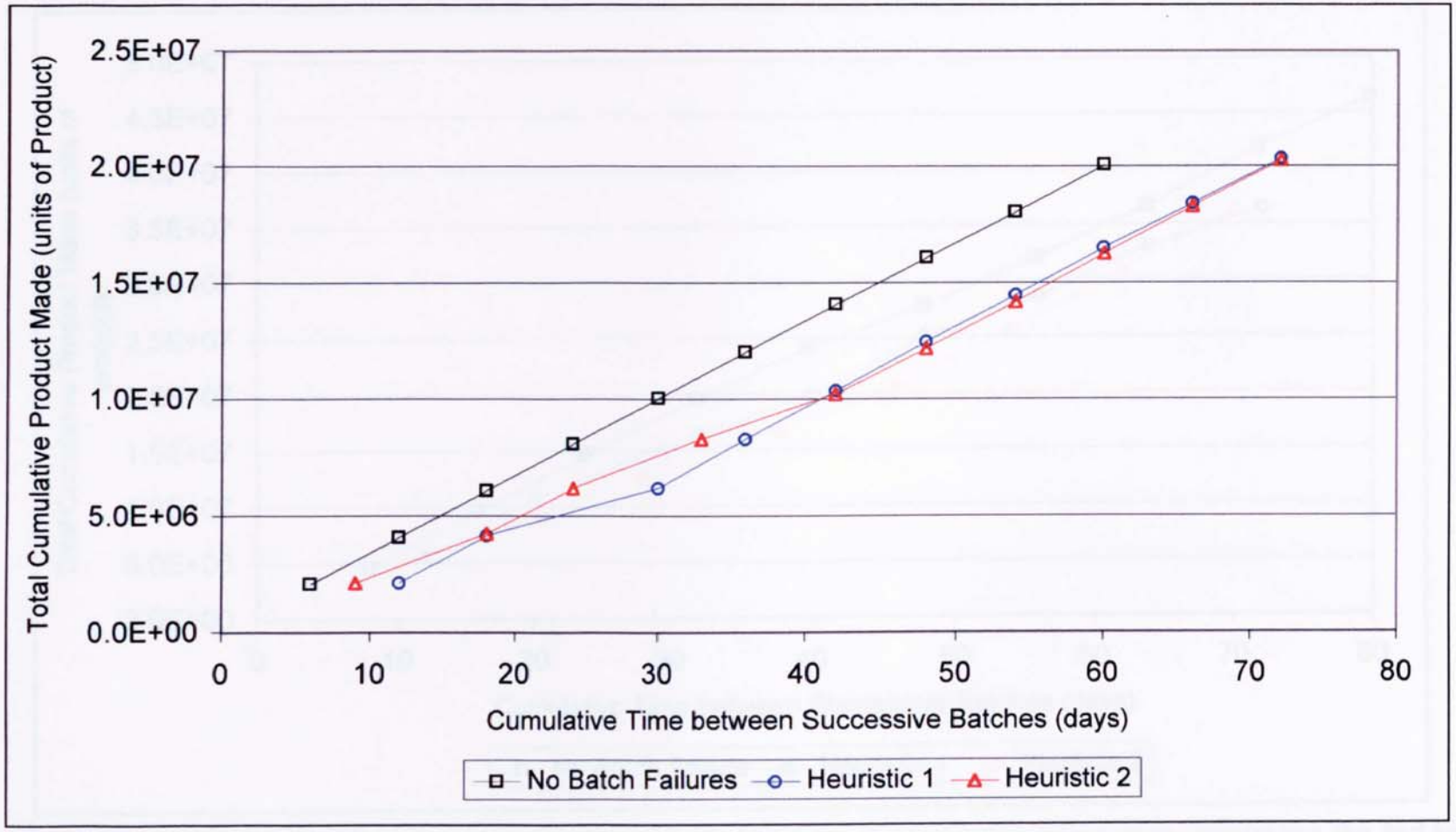

Figure 5.37 Profile comparing the total amount of product made between 3 production campaigns employing the batch {1S3 2P4} operating schedule, where 2 of the campaigns have been subject to batch failures due to contamination. For both campaigns where batch failures have occurred, two different schedule recovery heuristics are evaluated. (Start time basis = 28 days).

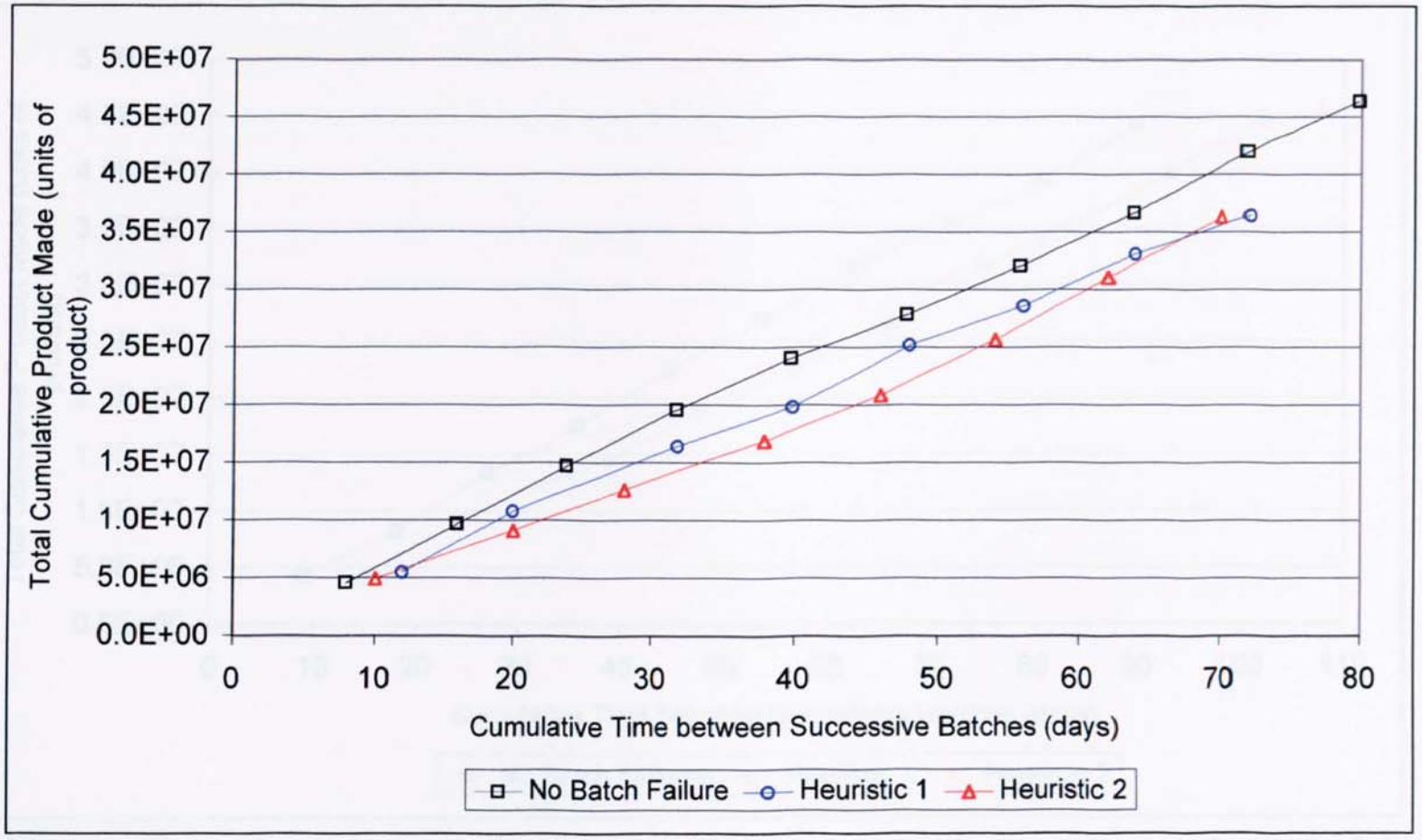

Figure 5.38 Profile comparing the total amount of product made between 3 production campaigns employing the fed-batch {1S2 2P7} operating schedule, where 2 of the campaigns have been subject to batch failures due to contamination. For both campaigns where batch failures have occurred, two different schedule recovery heuristics are evaluated. (Start time basis = 16 days)

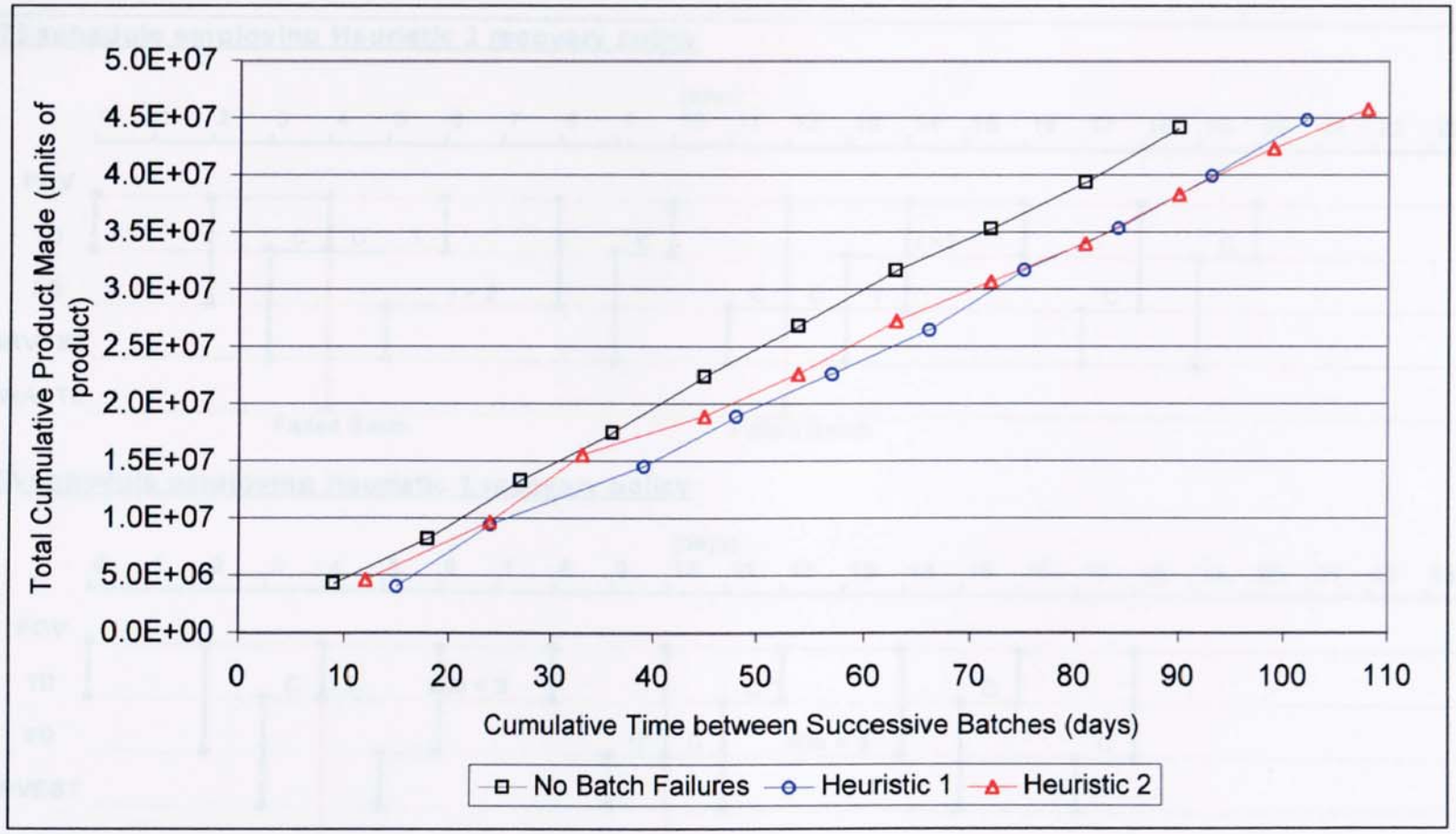

Figure 5.39 Profile comparing the total amount of product made between 3 production campaigns employing the fed-batch {1S3 2P7} operating schedule, where 2 of the campaigns have been subject to batch failures due to contamination. For both campaigns where batch failures have occurred, two different schedule recovery heuristics are evaluated. (Start time basis = 20 days).

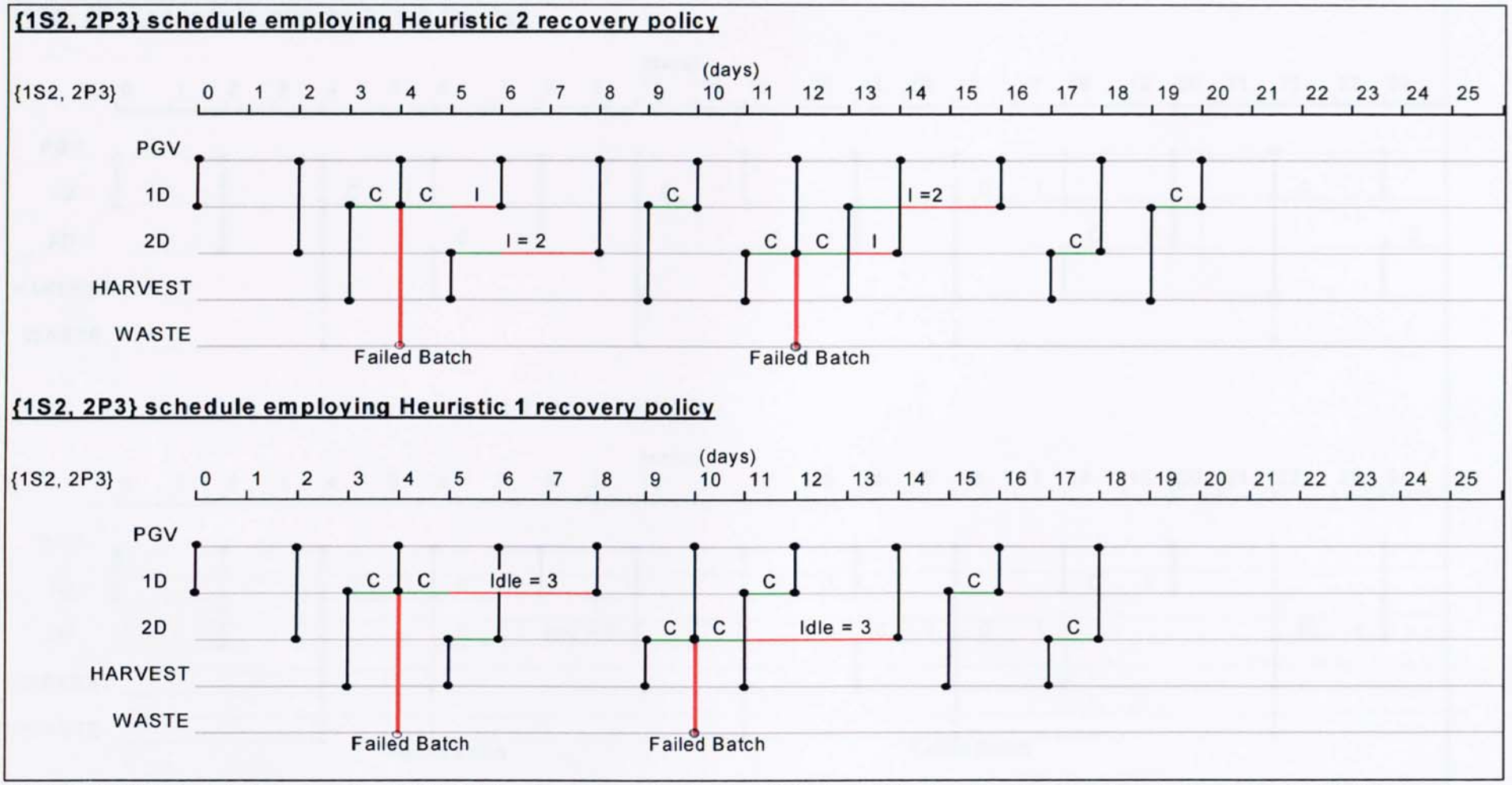

Figure 5.40 Network diagrams for {1S2, 2P3} schedule employing heuristic 1 and 2, showing the 'knock on' effect of a batch failure of one production vessel upon the idle time of the other production vessel when heuristic 2 is employed. (C = Cleaning & Sterilisation time, I = Idle time).

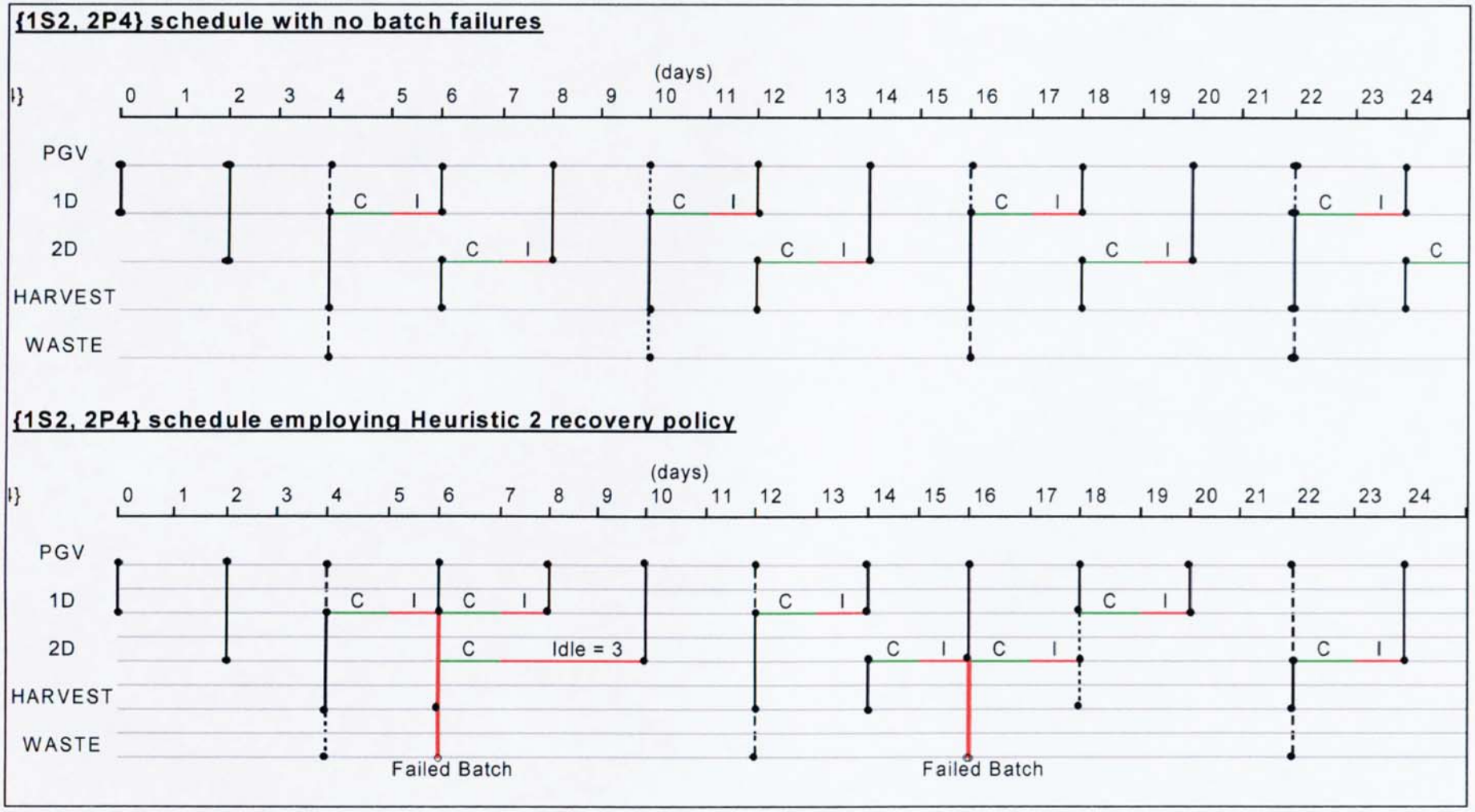

Figure 5.41 Network diagrams for the {1S2, 2P4} schedule, showing that the use of heuristic 2 eventually results in a schedule where the incurred idle times are the same as those incurred during normal operation without any batch failures.