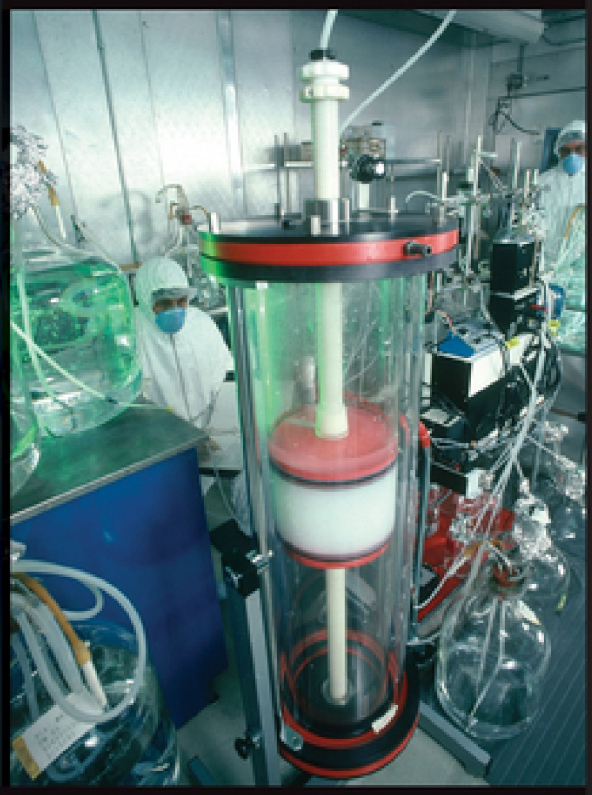

DOWNSTREAM INDUSTRIAL BIOTECHNOLOGY

Recovery and Purification

MICHAEL C. FLICKINGER, EDITOR

WILEY

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Recovery and Purification

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A JOHN WILEY & SONS, INC., PUBLICATION

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

Library of Congress Cataloging-in-Publication Data:

Encyclopedia of industrial biotechnology. Selections.
Downstream industrial biotechnology : recovery and purification / edited by Michael C. Flickinger.
pages cm
Includes bibliographical references and index.
ISBN 978-1-118-13124-4 (hardback)
1. Biotechnology–Encyclopedias. I. Flickinger, Michael C., editor of compilation. II. Title.
TP248.16.E533 2013
660.6–dc23
2012030526

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

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PREFACE

Downstream Industrial Biotechnology is a compilation of essential in depth articles, organized topically and listed in alphabetical format, for biopharmaceutical, bioprocess and biologics process scientists, engineers and regulatory professionals from the comprehensive seven volumes of the *Encyclopedia of Industrial Biotechnology*. Process development for the manufacture of complex biomolecules involves solving many scientific, compliance and technical problems quickly in order to support pilot, preclinical and clinical development, technology transfer and manufacturing start-up. Every organization develops new processes from accumulated process knowledge. Accumulated process knowledge has a very significant impact on accelerating the time to market (and reducing the financial resources required) of products manufactured using recombinant DNA and living microbes, cells, transgenic plants or transgenic mammals. However, when an entirely new upstream platform or downstream unit operation is needed, there are few books that will quickly provide the depth of industry-relevant background. *Downstream Industrial Biotechnology* can fill this void as an advanced desk reference. This volume includes relevant biology, protein purification and engineering literature with abundant process examples provide by industry subject matter experts (SMEs) and academic scholars. This desk reference will also be useful for advanced biomanufacturing students and professionals to quickly gain in depth knowledge on how to design processes (and facilities) capable of being licensed to manufacture enzymes, biopharmaceutical intermediates, human and veterinary biopharmaceuticals or vaccines. The opportunity is yours to leverage the combined knowledge from scores of industry professionals from around the world who have contributed to *Downstream Industrial Biotechnology* to reduce the time and cost to deliver engineered proteins, biomolecules and cost-effective biologics to the market and especially to millions of patients worldwide.

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PART I

INTRODUCTION

INTRODUCTION

Downstream biomanufacturing processes increase product concentration and purity, while decreasing process volume. Therefore, *decreasing process volume without loss of product* is essential to increase product purity, while at the same time eliminating product contaminants. The biochemistry of different products (peptides, proteins, hormones, low-molecular-weight metabolic intermediates, complex antigens etc.), all of which are liable to degradation, dictates that different separation methods be used to isolate and purify these products from contaminating biomolecules produced by the upstream process. Optimal downstream product yield is the yield of recovered product in the appropriate final biologically active form and purity. Purified but inactive product is a contaminant, reduces overall process yield, and may have serious consequences on clinical safety and efficacy. That is why downstream process design has the greatest impact on the overall biomanufacturing cost.

As product purity increases, more product can be lost to inactivation, nonspecific binding to equipment surfaces, binding to membranes, and chromatography media or by precipitation, thus decreasing the recovery of product. Because of these potential losses, each additional separation step may reduce overall yield. Therefore, downstream separation scientists and engineers are continually seeking to eliminate or combine unit operations to minimize the number of process steps in order to maximize product recovery at a specified concentration and purity.

Section II of *Downstream Industrial Biotechnology* includes detailed methods used for the initial steps of cell separation, cell disruption (for intracellular products), filter aids and adsorbents for rapid protein capture and initial volume reduction. Each of these steps is critically affected by upstream process design (volume, product concentration, and contaminants derived from the growth media or host cells), which impacts every subsequent step of downstream product recovery and purification. In particular, cell separation and cell disruption methods can have a dramatic effect on contributing (or minimizing) contaminants such as nucleic acids, host cell proteins, cell membrane fragments or pyrogenic lipopolysaccharides that need to be removed from the final product in subsequent separation steps.

Although each upstream process decision impacts downstream product recovery and purification, not all contaminants come from upstream operations. In some cases contaminants can also be generated by downstream operations, as inactivated product (due to heating, proteolysis, photoinactivation or precipitation), bioburden or microbial contamination introduced during downstream operations (from the environment, water, operations staff etc.) or contaminants derived from materials in direct contact with the product (extractable, leachable contaminants).

The downstream steps described in Section III are optimized by absorbent surface area, selectivity, binding capacity, and degree of volume reduction to purify product in the concentration range needed for each subsequent step to meet overall criteria of scale, stability, purity, and potency. Therefore, close integration of the characteristics of the upstream biological system that produces the product with the engineering and optimal performance of the downstream product separation, concentration. and purification operations are essential. This means that separation engineers, bioseparation and bioanalytical scientists, and manufacturing operations staff with broad expertise in working with labile biological molecules all need to work and communicate effectively as a team to design a downstream process that can be scaled from the

laboratory bench and transferred to the manufacturing scale. It also means that downstream process scientists must *continually provide feedback information to upstream* process engineers and scientists to minimize the impact of upstream changes (cell line changes, media composition changes, the addition of antifoam, degradation of product during in-process storage or holds) on downstream separation operations. Therefore, the companion volumes of *Upstream Industrial Biotechnology* should also be consulted when designing a downstream process.

Each downstream step requires process development and optimization (for purity, overall yield) because of the complexity of the structure of the biological molecules being purified and the complexity of contaminants. Section III also includes approaches for scale down of purification operations. Each downstream step is expensive to optimize at the pilot or manufacturing scale. This expense is not only due to the scale of the equipment and expense of the separation media, but also because of the large quantity of valuable product needed to carry out optimization studies at scale.

Downstream operations require specialized equipment designed for separation of proteins, peptides, virus, particulate antigens or low-molecular-weight biomolecules while minimizing product degradation. Sections IV and V focus on large scale equipment design and fluid transfer systems, and describe in detail many types of industrial bioseparation equipment. Of particular concern for products derived from mammalian cell lines are effective methods for virus inactivation and viral filtration that can be validated with model virus challenge. These methods are described in section V.

Not only do the upstream and downstream processes need to be designed to meet cGMPs and be capable of being licensed, but the facility used to carry out the process also must be designed so that it can be licensed. Section VI and VII of *Downstream* address facility design, facility validation, clean-in-place (CIP) and sterilization-in-place (SIP) methods. A major advance in facility design for downstream processes is the growing impact of single use (SU) disposable downstream materials and this is described in Section VI.

The overall goal of all downstream operations is not only to purify bulk product for formulation, but to achieve regulatory compliance and licensure so that final formulated and filled product can be released to consumers, physicians or patients. Section VII describes how Process Analytical Technology (PAT), bioburden testing and Quality by Design (QbD) impact downstream process design and contribute to regulatory compliance both for the USFDA and European regulatory agencies.

1

BIOPROCESS DESIGN, COMPUTER-AIDED

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1.1 INTRODUCTION

Bioprocess design is the conceptual work done prior to commercialization of a biological product. Given information on the potential market demand for a new product, bioprocess design endeavors to answer the following questions: What are the required amounts of raw materials and utilities for manufacturing a certain amount of product per year? What is the required size of process equipment and supporting utilities? Can the product be manufactured in an existing facility or is a new plant required? What is the total capital investment for a new facility? What is the manufacturing cost? How long does a single batch take? What is the minimum time between consecutive batches? During the course of a batch, what is the demand for various resources (e.g. raw materials, labor, and utilities)? Which process steps or resources are the likely production bottlenecks? What process and equipment changes can increase throughput? What is the environmental impact of the process? Which design is the "best" among several plausible alternatives?

Bioprocess design and project economic evaluation require the integration of knowledge from many different scientific and engineering disciplines. Design and evaluation are also carried out at various levels of detail. Table 1.1 presents a common classification of design and cost estimates and typical engineering costs for a $50 million capital investment project (1).

Order-of-magnitude estimates are usually practiced by experienced engineers who have worked on similar projects in the past. They take minutes or hours to complete, but the error in the estimate can be as high as 50%. Table 1.2 provides a good example of information typically employed for order-of-magnitude estimates of the capital investment for cell culture facilities. It lists capital investment for cell culture facilities of various sizes built in the last 10 years. The last column displays unit cost of capital investment expressed in millions of US dollars per cubic meter of production bioreactor capacity. The numbers range between 2.5 and 6.2 and for the more recent facilities the numbers are in the 5–6.2 range. Consequently, using the data of Table 1.2, one can safely estimate the capital investment for a new cell culture facility with production bioreactor capacity of 100 m^3 to be in the range of $500–650 million.

Engineers employed by operating companies usually perform level 2 and 3 studies. Such studies take days or weeks to complete using appropriate computer aids. The main objective of such a study is to evaluate alternatives and pinpoint areas of high cost and low yield. The results

Downstream Industrial Biotechnology: Recovery and Purification, First Edition. Edited by Michael C. Flickinger.

TABLE 1.1. Types of Design Estimates

Level	Type of Estimate	Accuracy	Cost ($1000)
1	Order-of-magnitude estimate (ratio estimate) based on similar previous cost data	≤50%	—
2	Project planning estimate (budget estimation) based on knowledge of major equipment items	≤30%	20–40
3	Preliminary engineering (scope estimate) based on sufficient data to permit the estimate to be budgeted	≤25%	50–100
4	Detailed engineering (capital approval stage) based on almost complete process data	≤15%	100–200
5	Procurement and construction (contractor's estimate) based on complete engineering drawings, specifications, and site surveys	≤10%	3000–7000

TABLE 1.2. Capital Investment for Cell Culture Facilities

Company	Capacity (m^3)	Completion Year	Investment ($ million)	Unit Cost ($ million/m^3)
Genentech	$8 \times 15 = 120$	2001	300	2.5
Amgen	$8 \times 8 = 64$	2002	300	4.7
Wyeth	$6 \times 15 = 90$	2003	325	3.6
Biogen Idec	$6 \times 15 = 90$	2005	450	5.0
BMS	$6 \times 20 = 120$	2009	750	6.2

are used to plan future research and development and to generate project budgets.

Level 4 and 5 studies are usually performed by engineering and construction companies that are hired to build new plants for promising new products that are at an advanced stage of development. These detailed estimates are beyond the scope of this chapter. Instead, the rest of this chapter will focus on level 2 and 3 studies. It should also be noted that opportunities for creative process design work are usually limited to preliminary studies. By the time detailed engineering work is initiated, a process is more than 80% fixed. Furthermore, most of the important decisions for capital expenditure and product commercialization are based on results of preliminary process design and cost analysis. This is why it is so important for a new engineer to master the skills of preliminary process design and cost analysis.

1.2 BENEFITS FROM THE USE OF COMPUTER AIDS

Process design calculations are greatly facilitated by the use of computer aids, such as spreadsheets, process simulators, finite capacity scheduling (FCS), and other specialized tools. Use of appropriate computer aids allows the process design team to quickly and accurately redo the entire series of calculations with a different set of assumptions and other input data. The benefits from the use of such tools depend on the type of product, the stage of development, and the size of the investment. For commodity biological products such as biofuels, minimization of capital and operating costs are the primary benefits. For high-value biopharmaceuticals, systematic process development that shortens the time to commercialization is the primary motivation. Figure 1.1 shows a pictorial representation of the benefits from the use of computer aids at the various stages of the commercialization process.

1.2.1 Idea Generation

When product and process ideas are first conceived, process modeling tools are used for project screening, selection, and strategic planning on the basis of preliminary economic analyses.

1.2.2 Process Development

During this phase, the company's process development groups look into the various options available for synthesizing, purifying, characterizing, and formulating the final product. At this stage, the process undergoes constant change. Typically, a large number of scientists and engineers are involved in the improvement and optimization of individual processing steps. The use of process simulation tools at this stage can introduce a common language of communication and facilitate team interaction. A computer model of the entire process can provide a common reference and evaluation framework to facilitate process development. The impact of process changes can be readily evaluated and documented in a

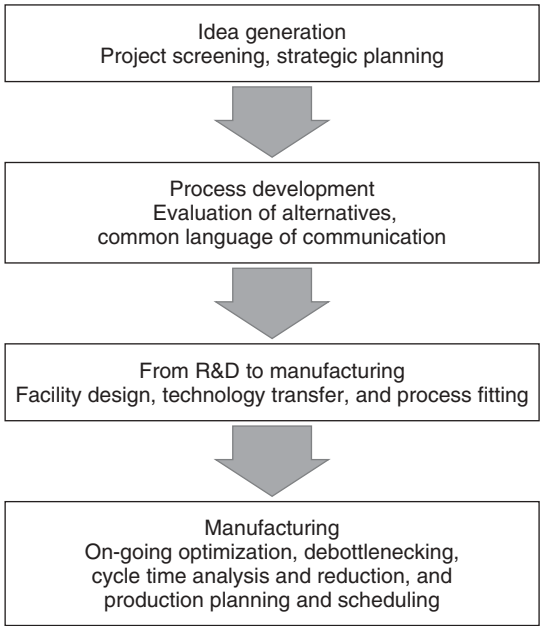

Figure 1.1. Benefits from the use of computer aids.

systematic way. Once a reliable model is available, it can be used to pinpoint the cost-sensitive areas of a complex process. These are usually steps of high capital and operating cost or low yield and production throughput. The findings from such analyses can be used to focus further lab and pilot plant studies to optimize those portions of the process. The ability to experiment on the computer with alternative process setups and operating conditions reduces the costly and time-consuming laboratory and pilot plant effort.

The environmental impact of a process is another issue that can be readily evaluated with computer models. Material balances calculated for the projected large-scale manufacturing reveal the environmental hot spots. These are usually process steps that use organic solvents and other regulated materials of high disposal costs. Environmental issues not addressed during process development may lead to serious headaches during manufacturing. This is especially true for biopharmaceuticals because after a process has been approved by the regulatory agencies, it is extremely costly and time consuming to implement process changes.

1.2.3 Facility Design and/or Selection

With process development near completion at the pilot plant level, simulation tools are used to systematically design and optimize the process for commercial production. Availability of a good computer model can greatly facilitate the transfer of a new process from the pilot plant to the large-scale facility. If a new facility needs to be built, process simulators can be used to size process equipment and supporting utilities, and estimate the required capital investment. In transferring production to existing manufacturing sites, process simulators can be used to evaluate the various sites from a capacity and cost point of view and select the most appropriate one. The same can apply to outsourcing of manufacturing to contract manufacturers.

1.2.4 Manufacturing

In large-scale manufacturing, simulation tools are mainly used for on-going process optimization and debottlenecking studies. Other computer aids that play an important role in manufacturing include FCS, manufacturing resource planning (MRP), and enterprise resource planning (ERP) tools. FCS tools play an important role in batch chemical manufacturing. They are used to generate production schedules on an on-going basis in a way that does not violate constraints related to the limited availability of equipment, labor resources, utilities, inventories of materials, and so on. FCS tools close the gap between ERP/MRP tools and the plant floor (2). Production schedules generated by ERP/MRP tools are typically based on coarse process representations and approximate plant capacities and, as a result, solutions generated by those tools may not be feasible, especially for multiproduct facilities that operate at high capacity utilization. This can often lead to late orders that require expediting and/or to large inventories in order to maintain customer responsiveness. "Lean manufacturing" principles, such as just-in-time production, low work-in-progress (WIP), and low product inventories cannot be implemented without good production scheduling tools that can accurately estimate capacity (3,4).

1.3 COMMERCIALLY AVAILABLE TOOLS

Process simulation programs, also known as process simulators, have been in use in the chemical and petrochemical industries since the early 1960s. Established simulators for those industries include: Aspen Plus and HYSYS from Aspen Technology, Inc. (Cambridge, MA), ChemCAD from Chemstations, Inc. (Houston, TX), and PRO/II from SimSci-Esscor, Inc. (Lake Forest, CA).

The above simulators have been designed to model primarily continuous processes and their transient behavior. Most biological products, however, are produced in batch and semicontinuous mode (5,6). Such processes are best modeled with batch process simulators that account for time-dependency and sequencing of events. Batches from Batch Process Technologies, Inc. (West Lafayette, IN) was the first simulator specific to batch processes. It was commercialized in the mid-1980s. All of its operation

models are dynamic and simulation always involves integration of differential equations over a period of time. In the mid-1990s, Aspen Technology (Cambridge, MA) introduced Batch Plus, a recipe-driven simulator that targeted batch pharmaceutical processes. Around the same time, Intelligen, Inc. (Scotch Plains, NJ) introduced SuperPro Designer. A unique feature of SuperPro is its ability to model batch as well as continuous processes (7).

Discrete-event simulators have also found applications in the bioprocessing industries. Established tools of this type include ProModel from ProModel Corporation (Orem, UT), Arena and Witness from Rockwell Automation, Inc. (Milwaukee, WI), Extend from Imagine That, Inc. (San Jose, CA), and FlexSim from FlexSim Software Products, Inc. (Orem, UT). The focus of models developed with such tools is usually on the minute-by-minute time-dependency of events and the animation of the process. Material balances, equipment sizing, and cost analysis tasks are usually out of the scope of such models. Some of these tools are quite customizable and third-party companies occasionally use them as platforms to create industry-specific modules. For instance, BioPharm Services, Ltd. (Bucks, UK) have created a module that runs on top of Extend and focuses on biopharmaceuticals.

MS Excel from Microsoft is another common platform for creating models for integrated processes that focus on material balances, equipment sizing, and cost analysis. Some companies have even developed models in Excel that capture the time-dependency of batch processes. This is typically done by writing extensive code (in the form of macros and subroutines) in VBA (Visual Basic for Applications) that comes with Excel. K-TOPS from Alfa Laval Biokinetics, Inc. (Philadelphia, PA) belongs to this category.

In terms of production scheduling, established tools include Infor SCM from Infor Global Solutions (Alpharetta, GA), Optiflex from i2 Technologies, Inc. (Irving, TX), SAP APO from SAP AG (Walldorf, Germany), ILOG Plant PowerOps from ILOG SA (Gentilly, France), Aspen SCM (formerly Aspen MIMI) from Aspen Technology, Inc. (Cambridge, MA), and so on. Their success in the biochemical industries, however, has been rather limited so far. Their primary focus on discrete manufacturing (as opposed to batch chemical manufacturing) and their approach to scheduling from a mathematical optimization viewpoint are some of the reasons for the limited market penetration.

SchedulePro from Intelligen, Inc. (Scotch Plains, NJ) is a new FCS tool that focuses on scheduling of batch and semicontinuous biochemical and related processes. It is a recipe-driven tool with emphasis on generation of feasible solutions that can be readily improved by the user in an interactive manner.

The rest of this chapter will address, through an illustrative example, the use of simulation and scheduling tools for evaluating and optimizing integrated biochemical processes. Analysis and assessment of additional bioprocesses can be found in the literature (8).

1.4 MONOCLONAL ANTIBODY EXAMPLE

Monoclonal antibodies (Mabs) are the fastest growing segment within the biopharmaceutical industry (9). More than 20 Mabs and Fc fusion proteins are approved for sale in the United States and Europe and approximately 200 Mabs are in clinical trials for a wide variety of indications (2). The market is predicted to grow by around 20% per year and reach $17 billion in 2008 (10).

The high-dose demand for several Mabs translates into annual production requirement for purified product in the metric ton range. Such a process is modeled and analyzed with SuperPro Designer in the rest of this chapter. Figure 1.2 displays the flow sheet of the overall process. The generation of the flow sheet was based on information available in the patent and technical literature combined with our engineering judgment and experience with such processes. The computer files for this example are available as part of the evaluation version of SuperPro Designer at the website www.intelligen.com/literature. Additional examples dealing with other biopharmaceuticals as well as commodity biological products are available at the same website.

To model an integrated process on the computer using SuperPro Designer, the user starts by developing a flow sheet that represents the overall process. The flow sheet is developed by putting together the required unit procedures (see the next paragraph for an explanation), and joining them with material flow streams. Next, the user initializes the flow sheet by registering the various materials that are used in the process and specifying operating conditions and performance parameters for the various operations.

Most biopharmaceutical processes operate in batch mode. This is in contrast to petrochemical and other high-throughput industries that use continuous processes. In continuous production, a piece of equipment performs the same action all the time. In batch processing, on the other hand, a piece of equipment goes through a cycle of operations. For instance, an inoculum preparation step (P-5 in SBR1) includes the following operations (Fig. 1.3): *SIP, SET UP, TRANSFER IN-1(media), TRANSFER IN-2 (inoculum), FERMENT (fermentation operation), TRANSFER OUT (emptying vessel), CIP (cleaning in place)*. In SuperPro, the set of operations that compose a processing step is called a *unit procedure* (as opposed to a unit operation). The individual tasks contained in a procedure (e.g. transfer in, Ferment, and CIP) are called *operations*.

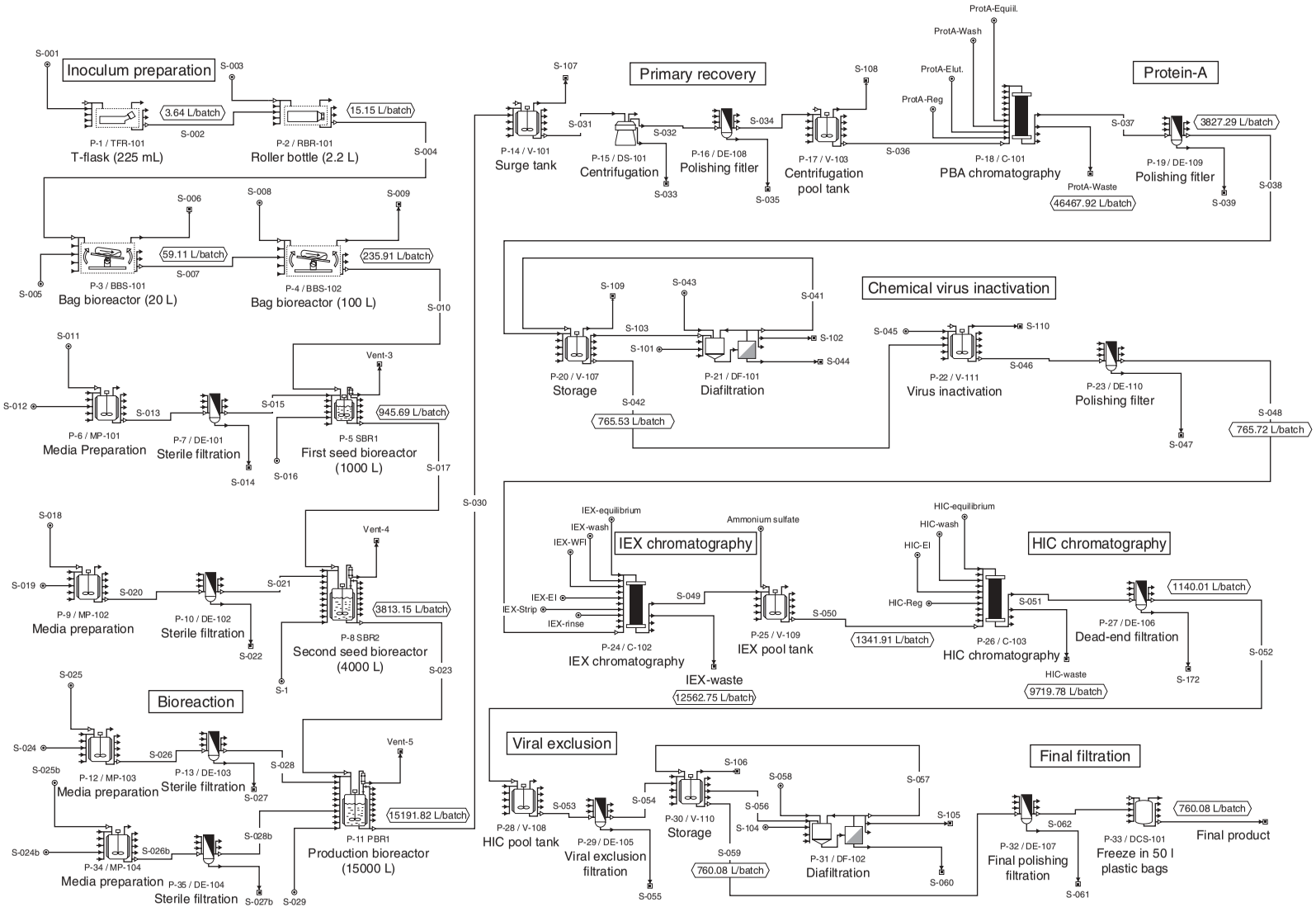

Figure 1.2. Monoclonal antibody production flow sheet.

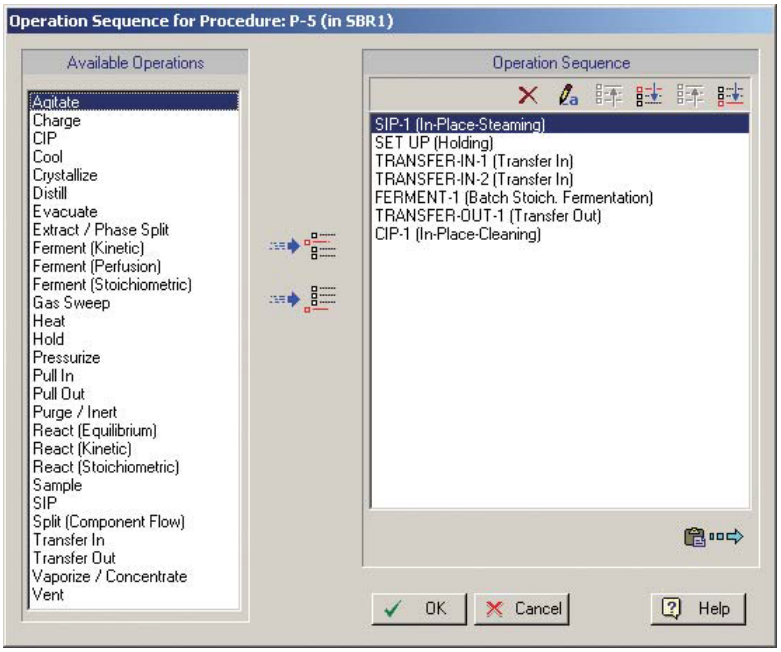

Figure 1.3. The operations associated with the P-5 unit procedure of Fig. 1.2. (This figure is available in full color at http://onlinelibrary.wiley.com/book/10.1002/9780470054581.)

A unit procedure is represented on the screen with a single equipment icon. In essence, a unit procedure is the recipe that describes the sequence of actions required to complete a single processing step. Figure 1.3 displays the dialog through which the recipe of a vessel unit procedure is specified. On the left-hand side of that dialog, the program displays the operations that are available in a vessel procedure; on the right-hand side, it displays the registered operations. The hierarchical representation of batch processes (also known as recipes) using unit procedures and operations is an approach that is recommended by the Instrument Society of America (ISA) because it facilitates modeling, control, and scheduling of batch operations (11).

For every operation within a unit procedure, the simulator includes a mathematical model that performs material and energy balance calculations. On the basis of the material balances, it performs equipment-sizing calculations. If multiple operations within a unit procedure dictate different sizes for a certain piece of equipment, the software reconciles the different demands and selects an equipment size that is appropriate for all operations. The equipment is sized so that it is large enough that it will not be overfilled during any operation, but it is no larger than necessary (to minimize capital costs). If the equipment size is specified by the user, the simulator checks to make sure that the vessel is not overfilled. In addition, the tool checks to ensure that the vessel contents will not fall below a user-specified minimum volume (e.g. a minimum stir volume) for applicable operations.

1.4.1 Process Description

1.4.1.1 Upstream. The upstream part is split in two sections: the inoculum preparation section and the bioreaction section. The inoculum is initially prepared in 225-mL T-flasks. The material is first moved to 2.2-L roller bottles, then to 20-L and subsequently to 100-L disposable bag bioreactors. Sterilized media is fed at the appropriate amount in all of these four initial steps (3.6, 11.4, 43.6, 175.4 kg/batch, respectively). The broth is then moved to the first (1000 L) and second (4000 L) seed bioreactor. For the seed bioreactors the media powder is dissolved in water for injection (WFI) in two prep tanks (MP-101 & MP-102) and then sterilized/fed to the reactors through 0.2-μm dead-end filters (DE-101 and DE-102). In the bioreaction section, serum-free low-protein media powder is dissolved in WFI in a stainless-steel tank (MP-103). The solution is sterilized using a 0.2-μm dead-end polishing filter (DE-103). A stirred-tank bioreactor (production bioreactor, PBR1) is used to grow the cells, which produce the therapeutic Mab. The production bioreactor operates under a fed batch mode. High media concentrations are inhibitory to the cells, so half of the media is added at the start of the process and the rest is fed at a variable rate during fermentation. The concentration of media powder in the initial feed solution is 17 g/L. The fermentation time is 12 days. The volume of broth generated per bioreactor batch is approximately 15,000 L, which contains roughly 22.6 kg of product (the product titer is approximately 1.5 g/L).

1.4.1.2 Downstream. Between the downstream unit procedures there are 0.2-μm dead-end filters to ensure sterility. The generated biomass and other suspended compounds are removed using a Disc-Stack centrifuge (DS-101). During this step, roughly 2% of Mab is lost in the solids waste stream resulting in a product yield of 98%. The bulk of the contaminant proteins are removed using a Protein-A affinity chromatography column (C-101). The following operating assumptions were made: (i) resin binding capacity is 15 g of product per liter of resin, (ii) the eluant or elution buffer is a 0.6% w/w solution of acetic acid and its volume is equal to 5 column volumes (CVs), (iii) the product is recovered in 2 CVs of eluant with a recovery yield of 90%, and (iv) the total volume of the solution for column equilibration, wash, and regeneration is 14 CVs. The entire procedure takes approximately 27 h and requires a resin volume of 362 L. The protein solution is then concentrated fivefold and diafiltered 2 times (in P-21/DF-101) using WFI as diluent. This step takes approximately 5 h and requires a membrane of 15 m^2. The product yield is 97%. The concentrated protein solution is then chemically treated for 1.5 h with Polysorbate 80 to inactivate viruses (in P-22/V-111). An ion exchange chromatography step follows (P-24/C-102). The following operating assumptions were made: (i) the resin's binding capacity is 40 g of product per liter of resin, (ii) a gradient elution step is used with a sodium chloride concentration ranging from 0.0 to 0.1 M and a volume of 5 CVs, (iii) the product is recovered in 2 CVs of eluant buffer with a yield on Mab of 90%, and (iv) the total volume of the solutions for column equilibration, wash, regeneration and rinse is 16 CVs. The step takes approximately 22.3 h and requires a resin volume of 158 L. Ammonium sulfate is then added to the ion exchange (IEX) eluate (in P-25/V-109) to a concentration of 0.75 M to increase the ionic strength in preparation for the hydrophobic interaction chromatography (HIC; P-26/C-103) that follows. The following operating assumptions were made for the HIC step: (i) the resin binding capacity is 40 g of product per liter of resin, (ii) the eluant is a sodium chloride (4% w/w) sodium di-hydrophosphate (0.3% w/w) solution and its volume is equal to 5 CVs, (iii) the product is recovered in 2 CVs of eluant buffer with a recovery yield of 90%, and (iv) the total volume of the solution for column equilibration, wash, and regeneration is 12 CVs. The step takes approximately 22 h and requires a resin volume of 142 L. A viral exclusion step (DE-105) follows. It is a dead-end type of filter with a pore size of 0.02 μm. This step takes approximately 2.3 h and requires a membrane of 1.45 m^2. Finally, the HIC elution buffer is exchanged for the product bulk storage (PBS) buffer and concentrated 1.5-fold (in DF-102). This step takes approximately 4 h and requires a membrane of 7 m^2. The approximately 580 L of final protein solution is stored in fifteen 50-L disposable storage bags (DCS-101). Approximately, 14.6 kg of Mab are produced per batch. The overall yield of the downstream operations is approximately 64.5%.

1.4.2 Material Balance

Table 1.3 provides a summary of the overall material balance of the process. Note the large amount of WFI utilized per batch. A major part of WFI is consumed for cleaning and buffer preparation. Approximately, 14.6 kg of Mab are produced per batch.

TABLE 1.3. Raw Material Requirements

	Requirement		
Raw Material	(kg/yr)	(kg/batch)	(kg/kg MP)
Inoculation Media	374	4.68	0.32
WFI	9,403,568	117,545	8,058
Phosphoric acid	44,113	551.41	37.80
Sodium hydroxide	34,164	427.05	29.28
Serum-free media	35,882	448.52	30.75
EDTA, sodium	2,544	31.80	2.18
Sodium chloride	53,600	670.00	45.93
TRIS base	1,272	15.90	1.09
TRIS HCl	3,815	47.69	3.27
Acetic acid	3,457	43.21	2.96
Sodium citrate	623	7.78	0.53
KCl	1	0.01	0.001
KH_2PO_4	1	0.01	0.001
Na_2HPO_4	1,817	22.72	1.56
NaH_2PO_4	105	1.31	0.09
Ammonium sulfate	8,104	101.31	6.94
Polysorbate 80	5	0.06	0.01
Total	9,593,440	119,918	8,221

MP, purified Mab.

1.4.3 Scheduling and Cycle Time Reduction

Figure 1.4 displays the Gantt chart of the process for four consecutive batches. The schedule represents a plant that has a single production train. The cleaning-in-place (CIP) skids can be seen at the top of the graph. The batch time is approximately 50 days. This is the time required from the start of inoculum preparation to the final product purification of a single batch. A new batch is initiated every 2 weeks (14 days). The production bioreactor (PBR1) is the time (scheduling) bottleneck. On an annual basis the plant processes 20 batches and produces approximately 292 kg of purified Mab. It is clear from the chart that under these conditions the downstream train is underutilized and the cycle time of the process—the time between consecutive batch starts—is relatively long. The cycle time of the process can be reduced and the plant throughput increased by installing multiple bioreactor trains that operate in staggered mode (out of phase) and feed the same purification train. Figure 1.5 represents a case where four bioreactor trains feed the same purification train. The new cycle time is 3.5 days, which is one-fourth of the original. Under these conditions, the plant processes 80 batches per year and produces approximately 1167 kg of Mab per year. Some biopharmaceutical companies have installed more than four bioreactor trains per purification train aiming at cycle times as low as 2 days.

1.4.4 Sizing of Batch Utilities

Another characteristic of batch processing is the variable demand of resources such as labor, utilities and raw materials as a function of time. Sizing of WFI systems is a common challenge during the design of new facilities and the retrofit of existing ones. WFI is used for preparing media and buffer solutions, for cleaning equipment, for generating clean steam, and so on. A WFI system consists of a distillation unit that generates the distilled water, a surge tank, and a circulation loop for delivering the material around the plant. The capacity may be limited by any of the following:

- The process can not, on average, consume more water than the still can generate.
- The process peak demand can not exceed the capacity of the circulation system.

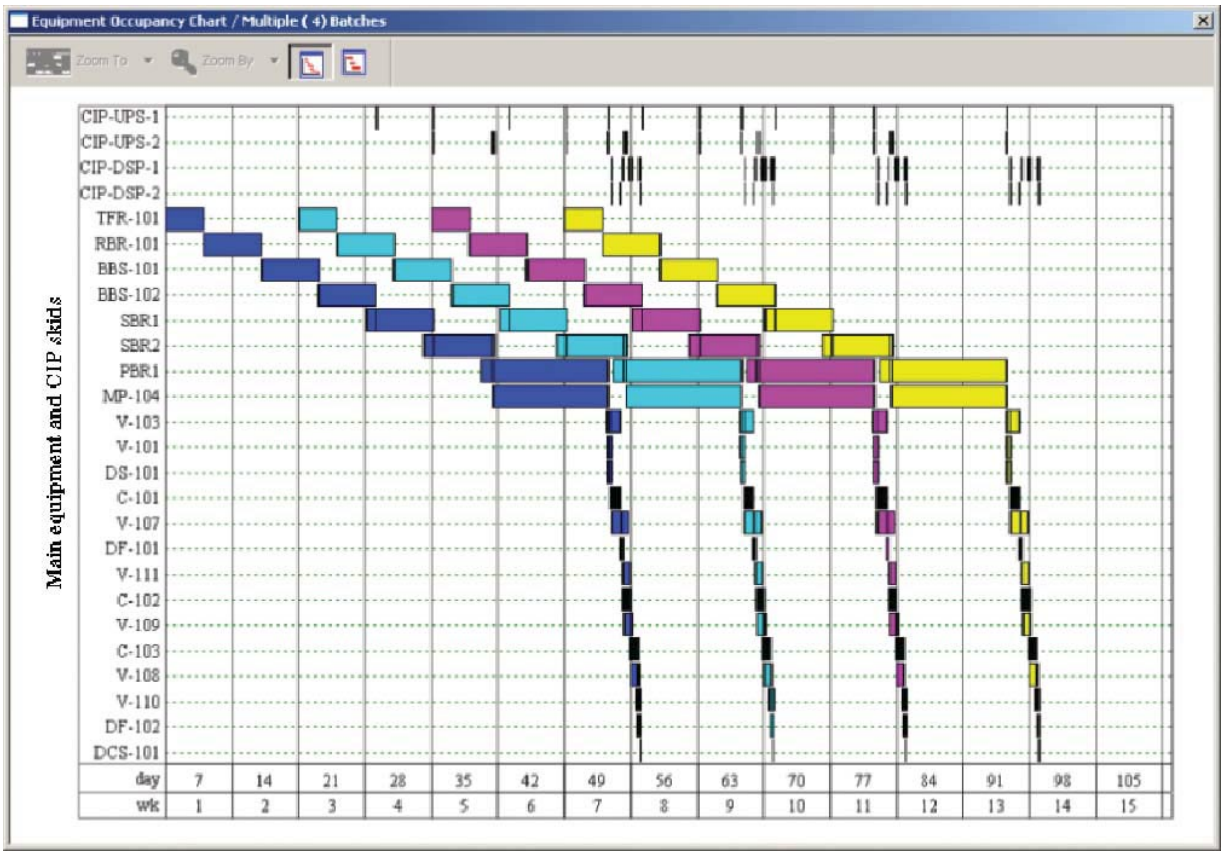

Figure 1.4. One bioreactor train feeding one purification train. (This figure is available in full color at http://onlinelibrary.wiley.com/book/10.1002/9780470054581.)

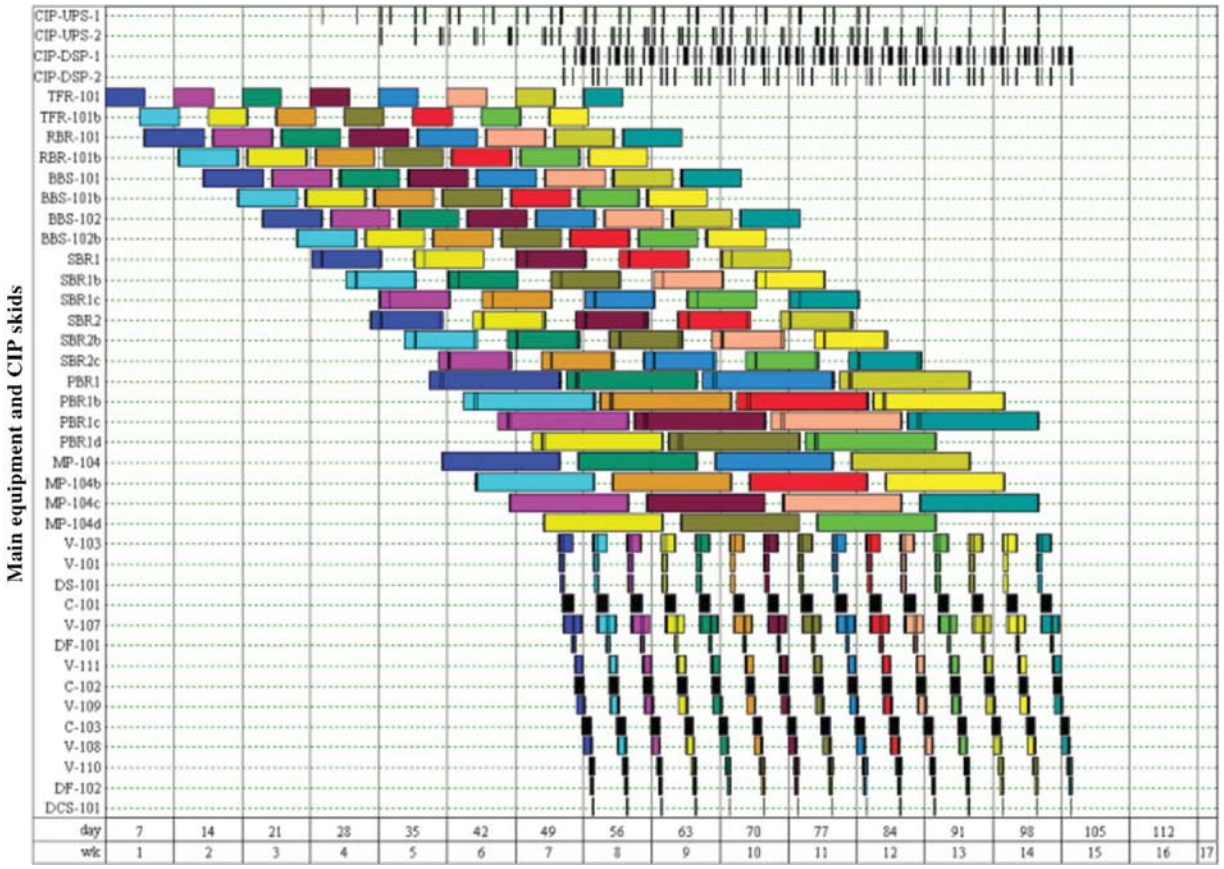

Figure 1.5. Four bioreactor trains feeding one purification train. (This figure is available in full color at http://onlinelibrary.wiley.com/book/10.1002/9780470054581.)

- The surge vessel must be large enough to maintain capacity during peak operation.
- In some plants, periodic sanitization cycles may interrupt all purified water draws.

Process modeling can provide reasonable estimates for the sizes of the still, the surge tank, and the pumping capacity of the circulation loop. Figure 1.6 displays the demand for WFI of the Mab process over time. The plots show the instantaneous and the 12-h average (heavy-line) demands. The chart also shows the 12-h cumulative amount that corresponds to the y-axis on the right. The peak instantaneous demand indicates the minimum pumping capacity for the system (11,500 kg/h or 50.7 gpm). The peak 12-h average rate provides an estimate for the capacity of still (1800 kg/h or 8 gpm), and the corresponding peak 12-h accumulation is an estimate of the surge tank capacity of 25,000 L. The trade-off between still rate and surge capacity can be examined by changing the averaging time. Selecting a longer period predicts a larger surge tank and a lower still rate.

Figure 1.7 displays the inventory profile of WFI in the surge tank for a tank size of 25,000 L and a still rate of 3500 L/h. The generation still is turned on when the level in the tank falls below 30% and it remains on until the tank is full. The operation rate of the still is depicted by the blue step-function lines. (The reader is requested to refer to the online version of this chapter for color indication.)

1.4.5 Economic Evaluation

Cost analysis and project economic evaluation are important for a number of reasons. For a new product, if the company lacks a suitable manufacturing facility with available capacity, it must decide whether to build a new plant or outsource the production. Building a new plant is a major capital expenditure (Table 1.2) and a lengthy process. To make a decision, management must have information on capital investment required and time to complete the facility. When production is outsourced, a cost-of-goods analysis serves as a basis for negotiation with contract manufacturers. A sufficiently detailed computer model can be used as the basis for the discussion and negotiation of the terms. Contract manufacturers usually base their estimates on requirements of facility/equipment utilization and labor per batch, which

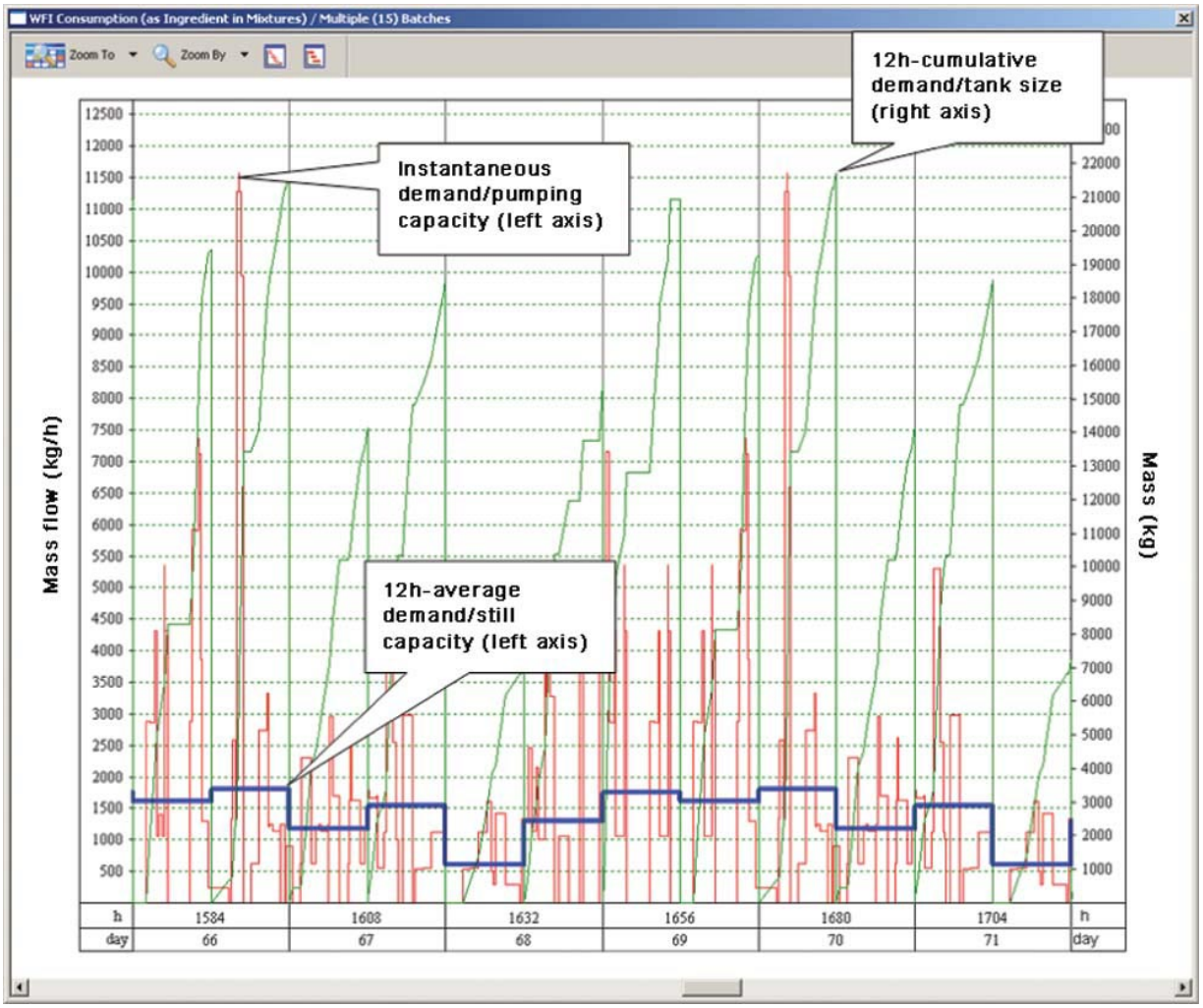

Figure 1.6. WFI demand as a function of time. (This figure is available in full color at http://onlinelibrary.wiley.com/book/10.1002/9780470054581.)

is information that is provided by a good model. SuperPro performs thorough cost analysis and project economic evaluation calculations. It estimates capital as well as operating cost. The cost of equipment is estimated using built-in cost correlations that are based on data derived from a number of vendors and literature sources. The fixed capital investment is estimated based on equipment cost and using various multipliers, some of which are equipment specific (e.g. installation cost) while others are process specific (e.g. cost of piping and buildings). The approach is described in detail in the literature (12–14). The rest of this section provides a summary of the cost analysis results for this example process.

Table 1.4 provides a list of major equipment items along with their purchase costs (generated by SuperPro Designer). The total equipment cost for a plant of this capacity (four production bioreactors each having a working volume of 15,000 L) is around $24 million. Approximately, a quarter of the equipment cost is associated with the four production bioreactors. The cost of vessels and filters that are seen in Fig. 1.2 but are missing from the table are accounted for under the "Cost of Unlisted Equipment" item. The economic evaluation also takes into account the vessels required for buffer preparation and holding that are not included in Fig. 1.2. A full model that includes all buffer preparation and holding activities and other advanced process modeling features can be downloaded from www.intelligen.com/literature.

Table 1.5 displays the various items included in the direct fixed capital (DFC) investment. The total DFC for a plant of this capacity is around $240 million or approximately 10 times the total equipment cost. The total capital investment that includes the cost of start-up and validation is around $300 million.