Bioprocess Design and Economics

by

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CHAPTER 11

Bioprocess Design and Economics

This chapter teaches students and practicing engineers the fundamentals of bioprocess design with an emphasis on bioseparation processes. It combines the information presented in earlier chapters for use in the context of integrated processes. The ultimate objective is to enable the reader to efficiently synthesize and evaluate integrated bioseparation processes.

Given a product and a desired annual production rate (process throughput), bioprocess design endeavors to answer the following and other related questions: What are the required amounts of raw materials and utilities needed for a single batch? What is the total amount of resources consumed per year? What is the required size of process equipment and supporting utilities? Can the product be produced in an existing facility or is a new plant required? What is the total capital investment? What is the manufacturing cost? What is the optimum batch size? How long does a single batch take? How much product can be generated per year? Which process steps or resources constitute scheduling and throughput bottlenecks? What changes can increase throughput? What is the environmental impact of the process (i.e., amount and type of waste materials)? Which design is the "best" among several plausible alternatives?

11.1 Instructional Objectives

After completing this chapter, the reader should be able to do the following:

- Initiate a process design and choose the appropriate sequencing of processing steps.
- Set up a process flowsheet using the unit procedure concept.
- Become familiar with batch process simulators.
- Schedule batch processes.
- Estimate capital and operating costs.
- Perform profitability analysis.
- Assess the environmental impact of a process.
- Perform process sensitivity analyses.

11.2 Definitions and Background

Process design is the conceptual work done prior to building, expanding, or retrofitting a process plant. It consists of two main activities, *process synthesis* and *process analysis.* Process synthesis is the selection and arrangement of a set of unit operations (process steps) capable of producing

the desired product at an acceptable cost and quality. Process analysis is the evaluation and comparison of different process synthesis solutions. In general, a synthesis step is usually followed by an analysis step, and the results of analysis determine the subsequent synthesis step.

Process design and project economic evaluation require integration of knowledge from many different scientific and engineering disciplines and are carried out at various levels of detail. Table 11.1 presents a common classification of design and cost estimates and typical engineering costs for a $50 million capital investment for a new plant.

TABLE 11.1

Types of Design Estimates, their Cost and Accuracy for a $50 Million Project [1]

Level	Type of Estimate	Error (%)	Cost ($1000)
1	Order-of-magnitude estimate (ratio estimate) based on similar previous projects	≤50	
2	Project planning estimate (budget estimation) based on knowledge of major equipment items	≤30	30–100
3	Preliminary engineering (scope estimate) based on sufficient data to permit the estimate to be budgeted	≤25	250–750
4	Detailed engineering (capital approval stage) based on almost complete process data	≤15	1250–2000
5	Procurement and construction (contractor's estimate) based on complete engineering drawings, specifications and site surveys	≤10	3500–7000

Figure 11.1 presents the need for design estimates of various types during the life cycle of product development and commercialization. The trapezoidal shape of the diagram represents the drastic reduction in product candidates as we move from feasibility studies to commercialization. In fact, the chances of commercialization at the research stage for a new product are only about 1 to 3%, at the development stage they are about 10 to 25%, and at the pilot plant stage they are about 40 to 60% [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 11.2 presents a sample of data typically used by experienced engineers for order-of-magnitude estimates. The table lists the capital investment for five large scale facilities built to manufacture therapeutic monoclonal antibodies using cell culture (by growing mammalian cells in stirred-tank bioreactors). Column #2 displays the

number of the production bioreactors, the working volume of each, and the total working volume. For instance, the Genentech facility in Oceanside (former Biogen Idec site) includes 6 production bioreactors each having a working volume of 15 m^3. Column #4 displays the total capital investment and column #5 displays the ratio of the total capital investment divided by the total production bioreactor volume. The ratio ranges from 3.3 to 6.2 with an average value of \$4.6 million per m^3 of bioreactor volume. Based on the data of Table 11.2, an engineer may conclude with some confidence that the capital investment for a new 100 m^3 (total production bioreactor volume) cell culture facility would be in the range of \$330 million to \$620 million and most likely around \$460 million. Please note, however, that advances in technology (e.g., cell lines that generate higher product titers and the use of single-use systems) and other factors may render such data obsolete and reduce the accuracy of order-of-magnitude estimates. As a result, cost estimates are progressively refined as new product candidates move through the development lifecycle shown in Figure 11-1.

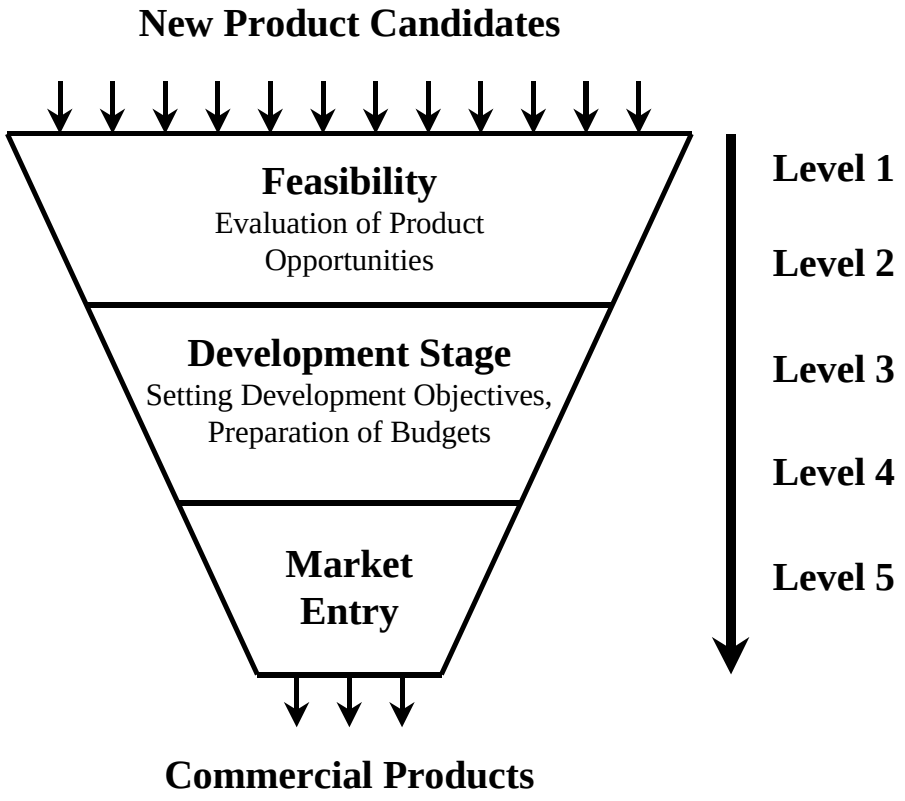

Figure 11-1 Types of design estimates during the lifecycle of a product [2].

Most engineers employed by operating companies usually perform level 2 and 3 studies. Such studies take weeks or months to complete using appropriate computer aids. The main objective of such studies is to evaluate alternatives and pinpoint the most cost-sensitive areas—the economic "hot spots"—of a complex process. The results of such analyses are used to plan future research and development and to generate project budgets.

TABLE 11.2

Capital Investments for Cell Culture Facilities

Company	**Bioreactor Capacity (m^3)**	**Completion Year**	**Investment ($ millions)**	**$ million per m^3**
Boehringer Ingelheim (Germany)	6x15 = 90	2003	296	3.3
Lonza Biologics (Portsmouth, NH)	3x20 = 60	2004	207	3.4
Genentech (Oceanside, CA)	6x15 = 90	2005	450	5.0
Bristol Myers Squibb (Devens, MA)	6x20 = 120	2009	750	6.2
Roche Pharmaceuticals (Switzerland)	6x12.5= 75	2009	375	5.0

Level 4 and 5 studies are usually performed by the engineering and construction companies hired to build new plants for promising new products that are at an advanced stage of development. Such estimates are beyond the scope of this chapter. Instead, the focus of the material in the rest of this chapter will be on level 1, 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 has been initiated, a process is more than 80% fixed. Furthermore, the vast majority of important decisions for capital expenditures and product commercialization are based on results of preliminary process design and cost analysis. This explains why it is so important for a new engineer to master the skills of preliminary process design and cost estimation.

Environmental impact assessment is an activity closely related to process design and cost estimation. Biochemical plants generate a wide range of liquid, solid, and gaseous waste streams that require treatment prior to discharge. The cost associated with waste treatment and disposal has skyrocketed in recent years due to increasingly strict environmental regulations. This cost can be reduced through minimization of waste generation at the source. However, generation of

waste from a chemical or biochemical process is dependent on the process design and the manner in which the process is operated. Thus, reducing waste in an industrial process requires intimate knowledge of the process technology. In contrast, waste treatment is essentially an add-on at the end of the process. In addition, minimization of waste generation must be considered by process engineers at the early stages of process development. Once a process has undergone significant development, it is difficult and costly to make major changes. Furthermore, regulatory constraints that are unique to the pharmaceutical industry restrict process modifications after clinical efficacy of the drug has been established. These are only some of the reasons that process synthesis and analysis must be initiated at the early stages of product development.

11.3 Synthesis of Bioseparation Processes

The development of a flowsheet for the recovery and purification of a biological product is a creative process that draws on the experience and imagination of the engineer. Attempts have been made to capture that experience on the computer in the form of expert systems [3–6] and automate to some extent the process synthesis tasks. Experienced engineers heavily rely on certain *rules of thumb,* also known as *heuristics,* for putting together the skeleton of a recovery and purification process [7]. A few such heuristics follow:

1. Remove the most plentiful impurities first.
2. Remove the easiest-to-remove impurities first.
3. Make the most difficult and expensive separations last.
4. Select processes that make use of the greatest differences in the properties of the product and its impurities.
5. Select and sequence processes that exploit different separation driving forces.

Figure 11.2 provides a generalized structure for creating an initial block diagram representation of a product recovery process. For each product category (intracellular or extracellular) several branches exist in the main pathway. Selection among the branches and alternative unit operations is based on the properties of the product, the properties of the impurities, and the properties of the producing microorganisms, cells, or tissues. Bioprocess synthesis thus consists of sequencing steps according to the five heuristics above and the structure of Figure 11.2. The majority of bioprocesses, especially those employed in the

production of high value and low volume products, operate in batch mode. Conversely, continuous bio-separation processes are utilized in the production of commodity biochemicals, such as organic acids and bio-fuels.

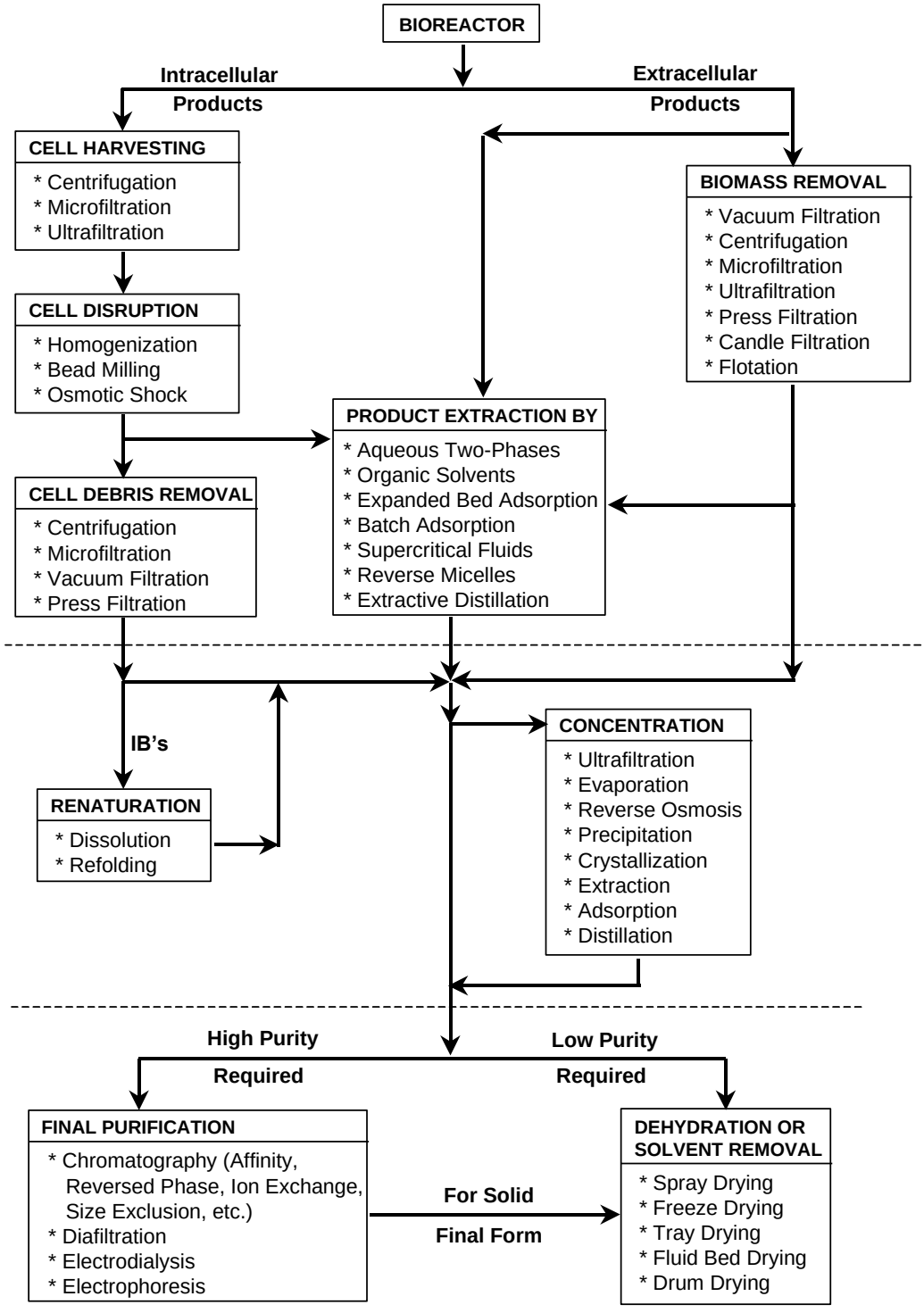

Figure 11-2 Generalized block diagram of downstream processing [5].

11.3.1 PRIMARY RECOVERY STAGES

Primary recovery encompasses the first steps of downstream processing where some purification and broth volume reduction occurs. Primary recovery includes both the solids separation stage and parts of the product isolation stages discussed in Chapter 1 (Table 1.9). According to Figure 11.2, the selection of the first step depends on whether the product is intracellular (remains inside the microorganism after its expression) or extracellular (secreted into the solution). Almost all low molecular weight bioproducts are extracellular, as are many that have a high molecular weight. Recovery and purification is easier for these bioproducts than for intracellular products because of the lower amount of impurities present. Most recombinant eukaryotic proteins produced by prokaryotic microorganisms are intracellular products (see Chapter 3 for definitions of prokaryotic and eukaryotic cells). These proteins accumulate inside the host cell in either native or denatured form; the denatured intracellular products often form insoluble inclusion bodies (IBs). A brief review of the most common primary recovery steps (described in Chapters 3, 4, 5, and 6) follows, and various rationales for unit operation selection are included. The human insulin process analyzed in this chapter provides additional information on the recovery and purification of intracellular products.

Recovery of Intracellular Products

Cell Harvesting The first purification step for intracellular products is cell harvesting. Removal of the extracellular liquid is in agreement with the first general heuristic: *Remove the most plentiful impurities first.*

As seen in Figure 11.2, centrifugation and or membrane filtration (either microfiltration or ultrafiltration) are the only techniques used for large-scale cell harvesting. As explained in Chapter 5, centrifugation has advantages for large and dense microorganisms (diameter >2 μm and density >1.03 g/cm^3). For instance, centrifugation is very efficient for harvesting yeast. For smaller microorganisms, various coagulation techniques can be used to increase the size of the settling particles (see Chapter 3). Membrane filtration has advantages for harvesting small and light cells. Another advantage of membrane filtration is its superior product recovery. Cell loss during centrifugation is typically 1 to 5%. However, with membrane filtration, essentially all cells are recovered unless there is cell disruption (lysis) or unless the membranes rip.

Cell Disruption The second step for intracellular products is usually cell disruption, which serves to break open the host cells and release the intracellular product. The various options for

cell disruption were presented in Chapter 3. Disruption of bacteria and yeast is carried out either by high pressure homogenizers or bead mills [8]. For large capacities (several cubic meters per hour) only high pressure homogenizers are practical. Osmotic shock is often used for release of periplasmic products that accumulate between the cell membrane and the cell wall.

Prior to disruption, the concentrate is often diluted (by 5–10%) with a "lysis buffer" to create conditions that minimize product denaturation upon release from the cell. For hard-to-disrupt microorganisms, multiple homogenization passes at 500 to 1000 bar are required. Multiple passes are also required if the product forms inclusion bodies. This allows the IBs to be released and breaks the cell debris into very small particles, facilitating the separation of IBs from cell debris further downstream. Some product degradation also occurs during cell disruption as a result of high shear at interfaces and oxidation.

Removal of Cell Debris The cell debris generated by cell disruption is usually removed by centrifugation or microfiltration. Other options include rotary vacuum filtration, press filtration, depth filtration, extraction, and expanded-bed adsorption (EBA) chromatography.

Soluble Product. When the product is soluble, it is recovered during cell debris removal either in the light phase of a centrifuge or in the permeate stream of a filter. Centrifuges are only able to efficiently separate fairly large particles of cell debris (>0.5 μm Stokes diameter). Therefore, when a centrifuge is used for cell debris removal, a polishing filtration step must follow the centrifugation in order to remove small debris particles that might otherwise cause severe problems in processes downstream such as chromatography. Filters of various types (e.g., depth, press, candle, rotary vacuum, membrane microfilters) can be used for polishing. Alternatively, these filters can be used for cell debris removal with no preceding centrifugation step. It is very difficult to predict a priori which filter will perform best for a specific product, so lab and pilot scale testing are typically used to make this decision. In addition, when microfilters are used for cell debris removal, some degree of diafiltration is required to achieve an acceptable product recovery yield.

Insoluble Product. When the product is insoluble and forms inclusion bodies, it must first be separated from the cell debris particles, then dissolved and refolded (see insulin example later in this chapter for additional information on the subject). Fortunately, inclusion bodies usually have a large diameter (0.3–1.0 μm) and high density (1.3–1.5 g/cm^3) [9] and can be separated from cell debris with a disk-stack centrifuge (Chapter 5). The inclusion bodies are recovered in the

heavy phase of the centrifuge, while most cell debris particles remain in the light phase. The heavy phase is usually re-suspended and re-centrifuged two or three times to reach a high degree of inclusion body purity. Resuspension in a solution of a detergent and/or a low concentration of a chaotropic agent is often practiced to facilitate the removal of other contaminants. The pH and the ionic strength of the solution are adjusted to reduce the hydrophobicity of the cell debris particles and to enhance their removal in the light phase. Final product purity exceeding 70% is quite common.

Product Extraction/Adsorption Separation of soluble product from cell debris can be carried out by extraction and/or adsorption. Organic solvents are commonly used as extractants for low molecular weight products, such as various antibiotics. Aqueous two-phase systems have found applications for recovery of proteins. The criteria for extractant selection are as follows: (a) the partition coefficient of the product should be higher than the partition coefficient of the contaminants, (b) the extractant should not degrade the product, (c) the extractant should not be expensive and should be easy to recover or dispose of (see Chapter 6 for more detailed information on extraction).

Alternatively, product separation from debris and simultaneous concentration can be achieved by adsorptive techniques [10]. Adsorbents of various types (e.g., ion exchange, reversed phase, affinity) can be used. This type of purification requires the disrupted cells and product to be mixed in a stirred tank with an adsorbent. A washing step, where most of the cell debris particles and contaminants are washed out, follows product adsorption. Expanded bed adsorption (EBA) chromatography is an alternative technology for separating proteins from cell debris particles [11]. The feed is pumped upward through an expanded bed. Target proteins are bound to the adsorbent while cell debris and other contaminants pass through. A washing step removes all weakly retained material. An elution step follows that releases and further purifies the product (see Chapter 7 for more detailed information on adsorption).

Recovery of Extracellular Products

Biomass Removal In agreement with the second generic heuristic (*Remove the easiest-to-remove impurities first*), biomass removal is usually the first step of downstream processing of extracellular products. This step can be accomplished by using one (or more) of the following unit operations: rotary vacuum filtration, disk-stack or decanter centrifugation, press filtration, microfiltration, ultrafiltration, and flotation. Since each unit operation has advantages and

disadvantages for different products and microorganisms, the selection of the best unit operation(s) for a given system can be difficult.

Rotary Vacuum Filtration. Rotary vacuum filtration, especially with precoat, is the classical method for removal of mycelial organisms [12]. Rotary vacuum filters can operate continuously for long periods of time (see Chapter 4). In addition, the filtrate flux in these units is usually higher than 200 L $m^{-2} h^{-1}$ and may reach 1000 L $m^{-2} h^{-1}$. The most important disadvantage of this type of unit is the problem with disposal of the mixture of filter aid and biomass. Filter aid is added in equal or higher amounts than biomass. Stringent environmental laws have made it costly to dispose of such solid materials. Therefore, if the disposal cost of filter aid is relatively high where a new plant is going to be built, alternative unit operations should be considered for biomass separation. However, if the disposal cost of filter aid is relatively low, a rotary vacuum filter is a good choice. The citric acid process, which is described later in this chapter, offers an example where rotary vacuum filtration is used for biomass removal.

Centrifugation. Disk-stack and decanter centrifuges are frequently used at large scale [13, 14]. Disk-stack centrifuges operate at higher rotational speeds and remove smaller and lighter microorganisms. However, with the use of flocculating agents, the decanter centrifuge performance improves, and choosing between the two types becomes more difficult. It appears that the only criterion being applied when disk-stack is chosen instead of decanter is the ability to remove small, light microorganisms. Centrifugation does not require filter aid, which is a significant advantage over rotary vacuum filtration. In general, the centrifuge paste contains 40 to 60% v/v extracellular liquid. To recover the product dissolved in that liquid, the paste is usually washed and re-centrifuged.

Membrane Filtration. With membrane filters (micro- and ultrafilters), the extracellular product passes through the membrane while biomass and other particulate components remain in the concentrate. Concentration is usually followed by diafiltration to prevent product degradation and/or to improve the performance of the subsequent step. (see Chapter 4 for more information on the mode of operation of membrane filters). Membrane filters are used for biomass removal mainly in recovery of low molecular weight products, such as antibiotics from mycelia. For high molecular weight products, gel layer formation often limits application to case in which the amount of solids is rather small (e.g. cell culture).

11.3.2 INTERMEDIATE RECOVERY STAGES

The primary recovery stages just described are followed by the intermediate stages, where the product is concentrated and further purified. Intermediate recovery has similarities to the product isolation stages discussed in Chapter 1 (Table 1.9). If the product is soluble, product concentration is usually the first step. If the product is denatured and insoluble, it is first dissolved and refolded and then concentrated and purified.

Product Concentration

After primary separation, the product is usually in a dilute solution. Volume reduction by concentration is in agreement with heuristics 1 and 2. Common concentration options include ultrafiltration, reverse osmosis, evaporation, adsorption, precipitation, extraction, and distillation.

Ultrafiltration Ultrafiltration is used extensively for protein solution concentration. The molecular weight cutoff of the membrane is selected to retain the product while allowing undesirable impurities (mainly low molecular weight solutes) to pass through the membrane. The low operating temperature and the purification achieved along with concentration are some of the advantages of ultrafiltration over evaporation. The typical operating transmembrane pressure is 2 to 5 bar and the average flux is 20 to 50 L m^{-2} h^{-1}.

Reverse Osmosis Membranes with smaller pore sizes are used for reverse osmosis filters. The process of reverse osmosis may be used when concentrating medium to low molecular weight products (e.g., antibiotics, certain amino acids).

Evaporation Thin-film rotating evaporators can operate at relatively low temperatures (40–50°C) under vacuum. These units compete in the market with ultrafiltration and reverse osmosis for concentrating both low and high molecular weight compounds. Unlike ultrafiltration, however, evaporation lacks the capability to provide purification during concentration. Advantages include the ability to concentrate to a higher final solids concentration and the ability to handle large throughputs [15].

Precipitation Precipitation is often used for concentration and purification. Blood protein fractionation (see Chapter 8) and citric acid production (see later: Section 11.6.1) constitute typical applications. Addition of salts, solvents, and polymers and changes in pH, ionic strength, and temperature are commonly used to selectively precipitate compounds of interest [16]. Precipitation often follows an extraction carried out in a polymer/salt (e.g., PEG and potassium phosphate) aqueous two-phase system. When the product is recovered in the polymer-rich phase,

precipitation is accomplished by addition of more polymer. It is important for economic reasons to recover and recycle the precipitating materials. Precipitation is also used to remove contaminants (i.e., nucleic acids) by adding Manganese Sulphate $MnSO_4$ and streptomycin sulfate.

Distillation The process of distillation is used for concentrating and purifying low molecular weight and volatile compounds, such as acetone, ethanol, butanol, acetic acid, etc.

Pervaporation This membrane-based process has found applications in bio-fuels for the dehydration of ethanol and other alcohols. One component from a liquid mixture selectively permeates through a membrane, driven by a gradient in partial vapor pressure and leaving the membrane as a vapor [17]. Dehydration of ethanol-water azeotropic solutions (around 90% ethanol) is facilitated by the use of hydrophilic membranes. Hydrophobic membranes are used for removal/recovery of small amounts of organics from aqueous solutions.

Product Renaturation

Eukaryotic proteins produced by prokaryotic microorganisms often form insoluble inclusion bodies in the host cell. Inclusion bodies can be dissolved rapidly by using solutions of strong chaotropes, such as 6 M guanidine hydrochloride or urea, in the presence of a reducing agent, such as 0.5 M 2-mercaptoethanol or 50 mM dithiothrietol [18]. The dissolved protein is then allowed to refold to its native conformation by removing the chaotropic agents through diafiltration, dilution, or chromatography, with final protein concentrations in the range of 10 to 100 mg/L. Dilution is necessary for minimizing intermolecular interactions, which occur during product refolding and can lead to product inactivation. Addition of small amounts of thiols such as reduced glutathione (1–5 mM) and oxidized glutathione (0.01–0.5 mM) and incubation at 35 to 40°C for 5 to 10 h completes the re-folding process. Thus, choosing an upstream process that forms IBs entails consideration of the large volumes, hence large waste streams that are produced. More information on IB solubilization and protein refolding can be found in the insulin example (see later: Section 11.6.2) and also in “Solubilization and Refolding of Proteins in Inclusion Bodies” (Section 1.4.8).

11.3.3 FINAL PURIFICATION STAGES

The final purification steps are dependent on the required final product purity. Final purification includes both the purification and polishing stages discussed in Chapter 1 (Table 1.9). Pharmaceutical products require high purity, while industrial products require lower purity. For

products of relatively low purity, such as industrial enzymes, the final purification step is dehydration or more generally a solvent removal step. For high purity products, the final purification stages usually involve a combination of chromatographic and filtration steps [19]. If the final product is required in solid form, a dehydration or solvent removal step follows.

Chromatography

Chromatography is typically done later in a process in agreement with the third generic heuristic (*Make the most difficult and expensive separations last*). With the preceding separation steps, a large fraction of contaminants is removed, thereby reducing the volume of material that needs to be treated further. A sequence of chromatographic steps is usually required to achieve the desired final product purity, and the fourth and fifth generic heuristics are good guides for selecting and sequencing such steps [20]. For instance, according to the fifth heuristic, an ion exchange step should not be followed by another step of the same type. Instead, it should be followed by a reversed phase, affinity, or any other chromatography type that takes advantage of a different separation driving force.

Membrane adsorption units combine the high flux of membrane filters with the selective binding of chromatographic resins [21, 22]. As a result, they have advantages compared to traditional column chromatography when operated in flow-through mode for removing small amounts of specific contaminants. The membrane retains certain impurities (e.g., DNA molecule fragments) while the product molecules pass through the membrane. Such membrane systems are typically used as the last step of biopharmaceutical protein purification processes.

Simulated moving bed (SMB) chromatography is the method of choice for handling large volumes of material [23, 24]. It has found applications in the purification of amino acids, high-fructose corn syrup, cheese whey proteins, lactic acid, succinic acid, etc. In SMB systems, multiple columns operate out of phase using a complex system of valves. The feed stream usually passes through two columns, resulting in increased yield and resolution. One column is always out of use for cleaning. SMB systems can handle feed streams of continuous flow which is the preferred method of operation for the production of high-volume biochemicals.

Membrane filtration steps are commonly employed between chromatographic steps to exchange buffers and concentrate the dilute product solutions. See Chapter 7 for detailed information on chromatographic separation methods and Chapter 4 for the intervening membrane filtration steps. The insulin and monoclonal antibody examples presented later in this

chapter provide additional information on selection and operation of chromatographic separation units.

Crystallization and Fractional Precipitation

Crystallization and fractional precipitation can sometimes result in significant purification. Because these processes are cheaper to operate than chromatography, they should always be considered. The crystalline form of a bioproduct is especially advantageous, since the purity can be quite high and crystals can usually be stored for long periods of time. See Chapters 8 and 9 for a detailed discussion and analysis of precipitation and crystallization. The citric acid process which is analyzed later in this chapter is a good example of a product that is recovered and purified using precipitation and crystallization.

Dehydration or Solvent Removal

Dehydration or solvent removal is achieved with dryers. Spray, fluidized-bed, and tray dryers are used when products can withstand temperatures of 50 to 100°C. Freeze dryers are used for products that degrade at high temperatures. Freeze dryers require high capital expenditures and should be avoided if possible. See Chapter 10 for detailed information on product drying.

11.3.4 PAIRING OF UNIT OPERATIONS IN PROCESS SYNTHESIS

Besides using rules of thumb, or heuristics, for synthesizing bioseparation processes, it is often advantageous to consider how two unit operations can be paired to improve process efficiency. The following section lists some examples of operations that are logical to pair.

Extraction and Precipitation

The bioproduct is extracted with a solvent and then precipitated. To increase the yield, it is often desirable to concentrate the extract before the precipitation. The major hurdle to overcome for this pairing is to find a solvent that will work with both extraction and precipitation.

Precipitation and Hydrophobic Interaction Chromatography

The pairing of precipitation and hydrophobic interaction chromatography is usually accomplished for protein purification by using ammonium sulfate to precipitate impurities, leaving the desired bioproduct in the mother liquor. The ammonium sulfate is added to a concentration just below that needed to precipitate the bioproduct. After removal of precipitated impurities, the mother liquor can be applied directly to a hydrophobic interaction chromatography column, which was equilibrated to the concentration of ammonium sulfate in the mother liquor prior to the loading. The bioproduct adsorbs to the column under these

conditions. The column is eluted with a reverse gradient of ammonium sulfate, and the desired bioproduct is recovered in a fraction from the elution.

Filtration and Extraction

When the bioproduct is contained in the filtrate after filtration, it can often be extracted with an immiscible solvent. For the extraction of small molecules such as antibiotics with organic solvents, the pH must usually be adjusted to obtain the bioproduct in either its free base or free acid form so it will partition into the organic phase. For the aqueous two-phase extraction of proteins, two polymers or a salt and a polymer must be added. If the additions to the filtrate can be made in-line, the filtration and extraction steps can be carried out simultaneously, reducing the processing time.

11.4 Process Analysis

The flowsheets created during process synthesis must be analyzed and compared on the basis of capital investment, manufacturing cost, environmental impact, and other criteria to decide which ideas to consider further. Methodologies for estimating capital investment and manufacturing cost are presented in the next section of this chapter. In both cases, estimation is based on the results of material and energy balances and equipment sizing. These calculations are typically done using spreadsheets or process simulators. These tools allow the process design team to characterize a processing scenario, and then quickly and accurately redo the entire series of calculations for a different set of assumptions and other input data.

11.4.1 SPREADSHEETS

Spreadsheet applications, such as Microsoft Excel, have become as easy to use as word processors and graphics packages. In its simplest form, a spreadsheet is an electronic piece of paper with empty boxes, known as cells. The user can enter data in those cells, perform calculations, and generate results. Results from spreadsheets can be easily plotted in a variety of graphs.

11.4.2 PROCESS SIMULATORS AND THEIR BENEFITS

Process simulators are software applications that enable the user to readily represent and analyze integrated processes. They have been in use in the petrochemical industries since the early 1960’s. Established simulators for those industries include: Aspen Plus and HYSYS from Aspen Technology, Inc. (Burlington, MA), ChemCAD from Chemstations, Inc. (Houston, TX), and PRO/II from SimSci-Esscor, Inc. (Lake Forest, CA).

The simulators mentioned above have been designed to model primarily continuous processes and their transient behavior. Most biological products, however, are produced in batch and semi-continuous mode [25, 26]. Such processes are best modeled with batch process simulators that account for time-dependency and sequencing of events. The first simulator designed specifically for batch processes was called Batches (from Batch Process Technologies in West Lafayette, IN). It was commercialized in the mid 1980's. All of its operation models are dynamic and simulation always involves integration of differential equations over a period of time. In the mid 1990's, Aspen Technology (Burlington, MA) introduced Batch Plus (now called Aspen Batch Process Developer), a recipe-driven simulator that targeted batch pharmaceutical processes. Around the same time, Intelligen, Inc. (Scotch Plains, NJ) introduced SuperPro Designer [27, 28]. SuperPro Designer is a flowsheet-driven simulator which handles material and energy balances, equipment sizing and costing, economic evaluation, environmental impact assessment, process scheduling, and debottlenecking of batch and continuous processes.

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.

The benefits from the use of process simulators depend on the type of product, the stage of development and the size of the investment. For commodity biological products, such as bio-fuels, 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 11-3 shows a pictorial representation of the benefits from the use of such tools at the various stages of the commercialization process.

Idea Generation When product and process ideas are first conceived, process modeling tools are used for project screening, selection, and strategic planning based on preliminary economic analyses.

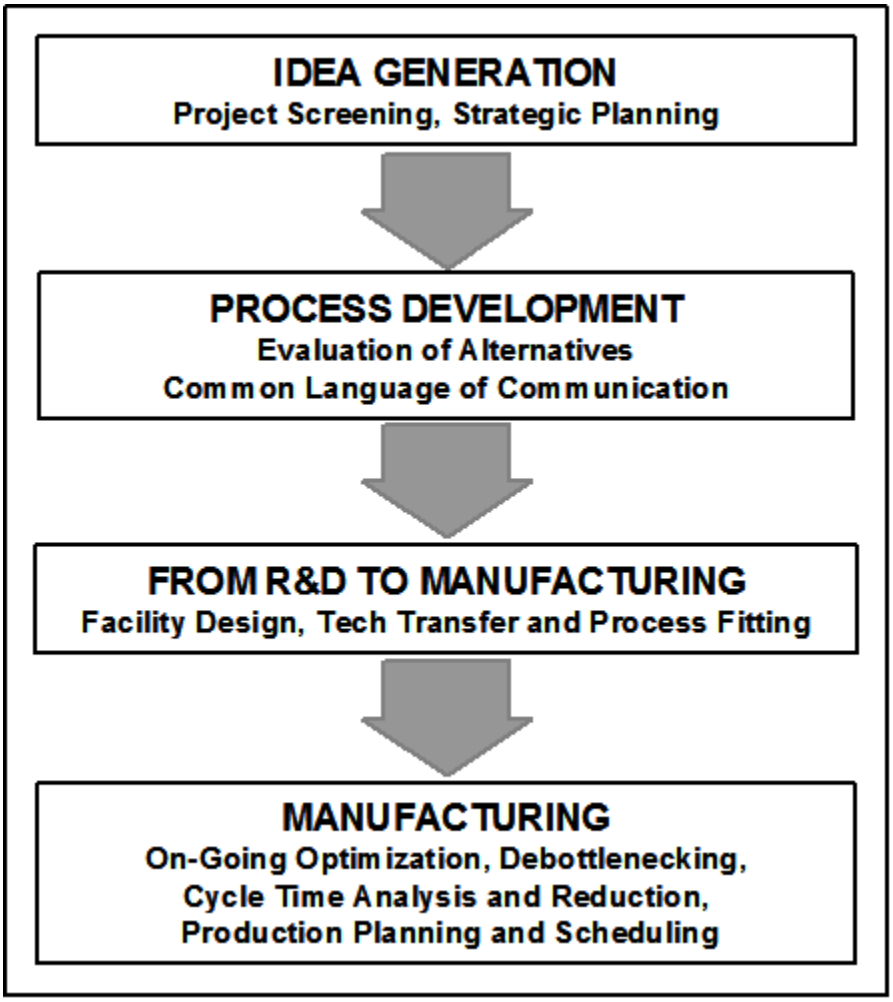

Figure 11-3 Benefits of using process simulators.

Process Development During this phase, the company's process development groups are looking into the various options available for synthesizing, purifying, characterizing, and formulating the final product. The process undergoes constant changes during development. Typically, a large number of scientists and engineers are involved in the improvement and optimization of individual processing steps. The use of process simulators 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 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

focus further lab and pilot plant studies in order 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. A simulator can also evaluate the environmental impact of a process. For instance, material balances calculated for the projected large scale manufacturing reveal environmental hot-spots. These are usually process steps that utilize organic solvents and other regulated materials with high disposal costs. Environmental issues which are not addressed during process development may lead to serious drawbacks during manufacturing.

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 size process equipment and supporting utilities, and estimate the required capital investment. In transferring production to existing manufacturing sites (technology transfer), process simulators can be used to evaluate the various sites from a capacity and cost point of view and select the most appropriate one.

Manufacturing In large scale manufacturing, simulation tools are mainly used for on-going process optimization and debottlenecking studies. Furthermore, tools that are equipped with batch process scheduling capabilities can be 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, etc.

11.4.3 USING A BIOCHEMICAL PROCESS SIMULATOR

The minimum requirements for a biochemical process simulator are the ability to handle batch as well as continuous processes and the ability to model the unit operations that are specific to bioprocessing. Because SuperPro Designer (from Intelligen, Inc.) has the ability to satisfy these requirements, we will use it to illustrate the role of such tools in bioprocess design. A functional evaluation version of SuperPro Designer and additional information on bioprocess simulation can be obtained at the website www.intelligen.com. Tutorial videos on the use of SuperPro Designer can be viewed at www.intelligen.com/videos.

To model an integrated process using a simulator, the user starts by developing a flowsheet

that represents the overall process. For instance, Figure 11.4 displays the flowsheet of a hypothetical process on the main window of SuperPro Designer. The flowsheet is developed by putting together the required unit operations (sometimes referred to as "unit procedures," as explained later in this section) and joining them with material flow streams. Next, the user initializes the flowsheet by registering (selecting from the component database) the various materials that are used in the process and specifying operating conditions and performance parameters for the various operations.

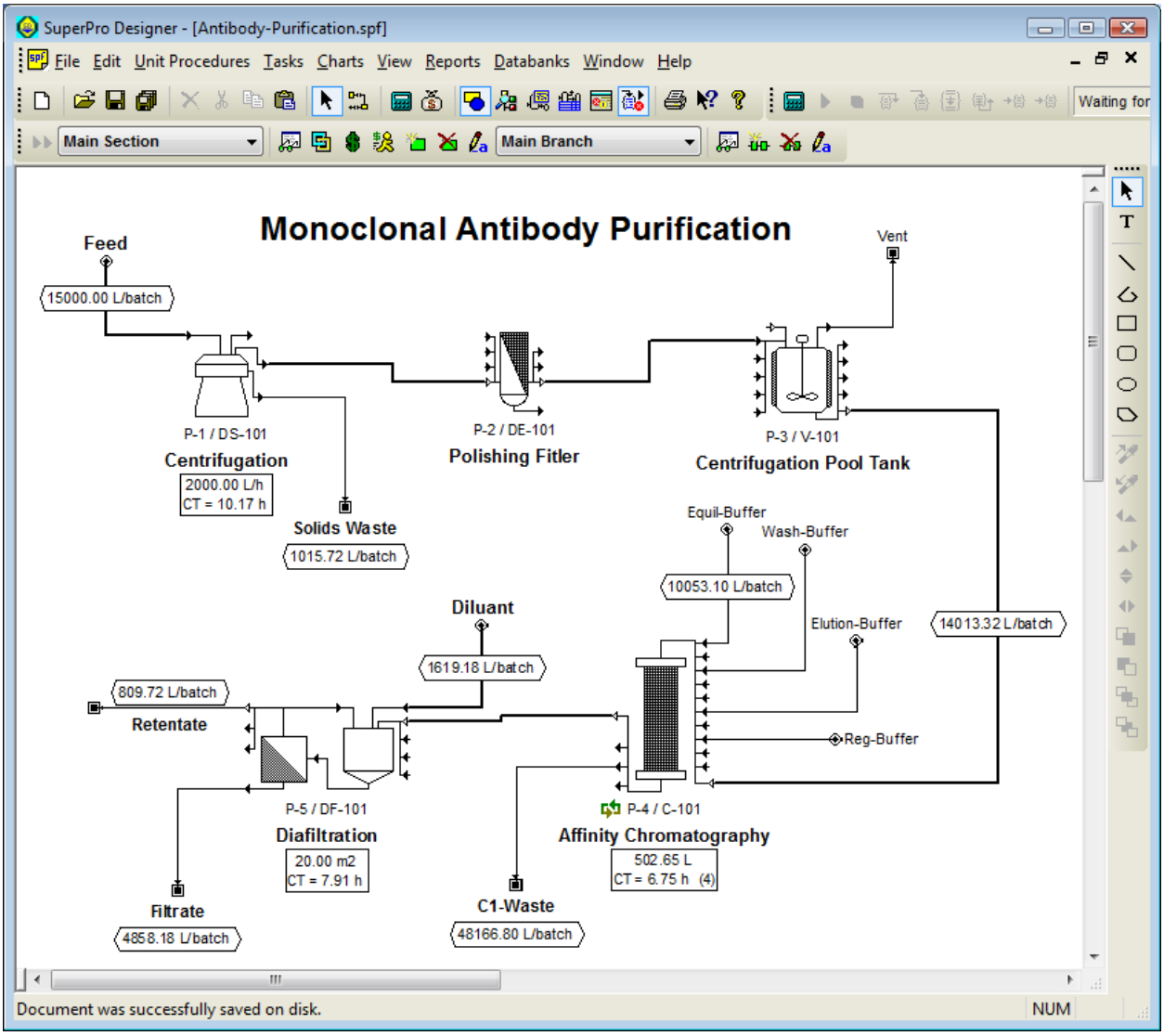

Figure 11-4 A flowsheet on the main window of SuperPro Designer.

Most biochemical processes operate in batch or semi-continuous mode. This is in contrast to continuous operation, which is typical in the petrochemical and other industries that handle large

throughputs. In continuous operations, a piece of equipment performs the same action all the time which is consistent with the notion of unit operations. In batch processing, on the other hand, a piece of equipment goes through a cycle of operations. For instance, a typical chromatography cycle includes *equilibration*, *loading*, *washing*, *elution*, and *regeneration* (Chapter 7). In SuperPro Designer, the set of operations that comprise a processing step is called a "unit procedure" (as opposed to a "unit operation"). Each unit procedure contains individual tasks (e.g., equilibration, loading) called operations. A unit procedure is represented on the screen with a single equipment icon (e.g., P-4 / C-101 in Figure 11.4 represents the affinity chromatography procedure). In essence, a unit procedure is the recipe of a processing step that describes the sequence of actions required to complete that step. Figure 11.5 displays the dialog through which the recipe of a chromatography unit procedure is specified. On the left-hand side of that dialog, the program displays the operations that are available in a chromatography procedure; on the right-hand side, it displays the registered operations (i.e. the operations that have been selected for this particular procedure). The significance of the unit procedure is that it enables the user to describe and model the various activities of batch processing steps in detail. Later in this chapter (in the examples, Section 11.6), we will see how the execution of these activities can be visualized as a function of time.

For every operation within a unit procedure, SuperPro includes a mathematical model that performs material and energy balance calculations. Based on the material balances, SuperPro performs equipment-sizing calculations similar to some of the homework problems in this book. 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. In other words, the equipment is sized to ensure that it will not be overfilled during any operation but is no larger than necessary (in order to minimize capital costs). In addition, the software checks to ensure that the vessel contents will not fall below a user-specified minimum volume (e.g., a minimum impeller volume) for applicable operations.

Before any simulation calculations can be done, the user must initialize the various operations by specifying operating conditions and performance parameters through appropriate dialog windows. For instance, Figure 11.6 displays the initialization dialog of a chromatography elution operation. Through this dialog, the user specifies the elution strategy (isocratic or gradient), selects the input and output streams, specifies the eluant volume on a relative (e.g., in

terms of bed volumes) or an absolute basis, specifies the fraction of buffer in which the product is recovered, specifies the linear velocity during elution, etc. Through the Labor tab of the same dialog window, the user provides information about labor requirements during this operation. Through the Scheduling tab, the user specifies the sequencing of this operation relative to another operation (either in the same procedure or in a different procedure) or relative to the beginning of the batch. After initialization of the operations, the simulator performs material and energy balances for the entire process and estimates the required sizes of equipment. Optionally, the simulator may be used to carry out cost analysis and economic evaluation calculations. The fundamentals of process economics are described in the next section, and pertinent examples are provided later in this chapter.

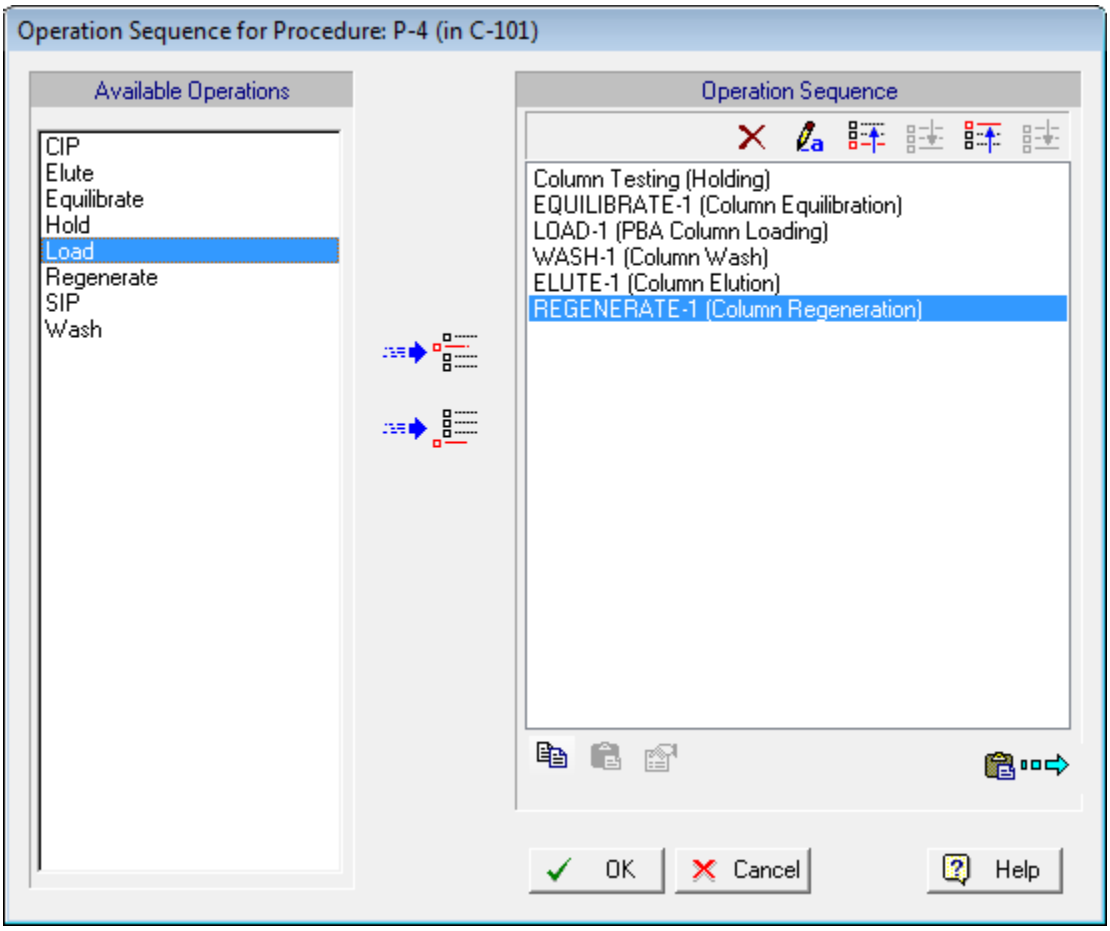

Figure 11-5 Window for adding operations to a unit procedure using SuperPro Designer.

Other tasks that can be handled by process simulators include process scheduling, environmental impact assessment, debottlenecking, and throughput analysis. Issues of process

scheduling and environmental impact assessment are addressed in Section 11.6. Throughput analysis and debottlenecking is the analysis of the capacity and time utilization of equipment and resources (e.g., utilities, labor, raw materials). The objective is to identify opportunities for increasing throughput with the minimum possible capital investment (see Section 11.6 for additional information on the subject).

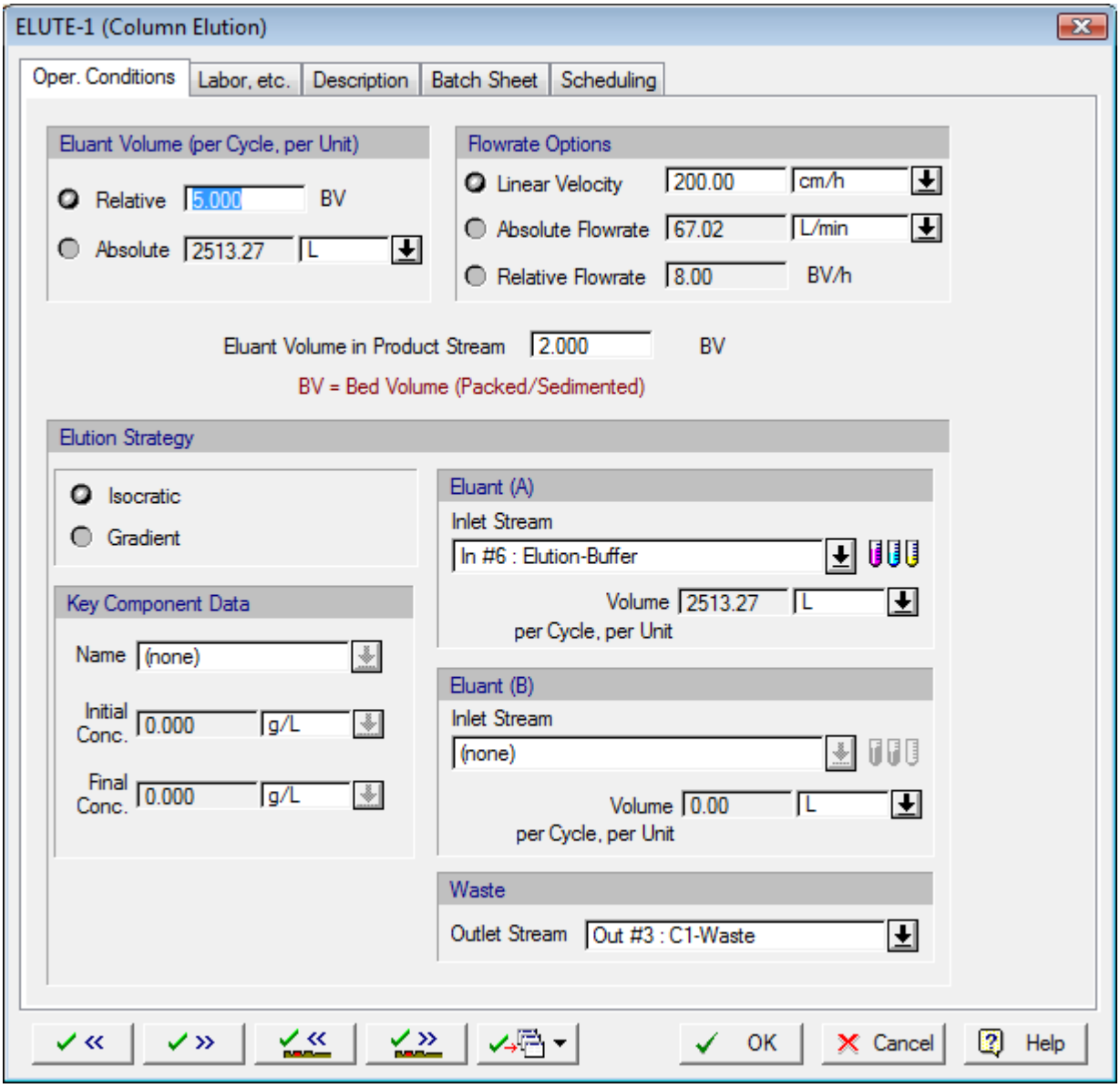

Figure 11-6 Dialog window of the elution operation.

Having developed a good model using a process simulator or a spreadsheet, the user may conduct virtual experiments with alternative process setups and operating conditions. This may potentially reduce costly and time-consuming laboratory and pilot plant effort. One must be

aware, however, that the GIGO (garbage in, garbage out) principle applies to all computer models. More specifically, if some assumptions and input data are incorrect, the outcome of the simulation will not be reliable. Consequently, validation of the model is necessary. In its simplest form, a review of the results by an experienced engineer can play the role of validation.

11.5 Process Economics

The preliminary economic evaluation of a project for manufacturing a biological product usually involves the estimation of capital investment, estimation of operating costs, and an analysis of profitability. For biopharmaceuticals, another figure worth considering is the average cost of new drug development, which is in the range of $500 to $1 billion [29, 30]. Much of this figure represents research and development (R&D) spending for all unsuccessful products. In other words, the actual average development cost per successful drug may be $50 to $100 million, but because more than 90% of new projects never reach commercialization, the average overall R&D cost skyrockets. This order-of-magnitude cost increase reinforces the need for effective process design tools and methodologies that assist engineers and scientists in efficiently evaluating and eliminating non-promising project ideas at the very early stages of product and process development.

11.5.1 CAPITAL COST ESTIMATION

The capital investment for a new plant includes three main items: direct fixed capital (DFC), working capital, and start-up and validation cost.

Direct Fixed Capital

The DFC for small to medium size biotechnology facilities is usually in the range of $50 to $200 million, whereas for large facilities it is in the range of $250 to $750 million. For preliminary design purposes, the various items of DFC are estimated based on the total equipment purchase cost (PC) using several multipliers sometimes called “Lang Factors”. Table 11.3 provides ranges and average values for the multipliers and a skeleton for the calculations. Detailed definitions of the various cost items and additional information can be found in traditional process design textbooks and the technical literature [1, 31–36].

Notice the wide range of multiplier values for estimating the cost of buildings. Plants for commodity biochemicals, such as ethanol and citric acid, fall on the low end of the range. Conversely, biopharmaceutical facilities with their expensive heating, ventilation, and air conditioning (HVAC) requirements fall on the high end. The average value of 0.45 corresponds

to relatively large plants that produce medium to high value products (e.g., industrial enzymes).

TABLE 11.3

Fixed Capital Cost Estimation

Cost item	Average multiplier	Range of multiplier values
Total plant direct cost (TPDC)		
Equipment purchase cost (PC)		
Installation	0.50 × PC	0.2–1.5
Process piping	0.40 × PC	0.3–0.6
Instrumentation	0.35 × PC	0.2–0.6
Insulation	0.03 × PC	0.01–0.05
Electrical	0.15 × PC	0.1–0.2
Buildings	0.45 × PC	0.1–3.0
Yard improvement	0.15 × PC	0.05–0.2
Auxiliary facilities	0.50 × PC	0.2–1.0
Total plant indirect cost (TPIC)		
Engineering	0.25 × TPDC	0.2–0.3
Construction	0.35 × TPDC	0.3–0.4
Total plant cost (TPC)	TPDC + TPIC	
Contractor’s fee	0.05 × TPC	0.03–0.08
Contingency	0.10 × TPC	0.07–0.15
Direct fixed capital (DFC)	TPC + Contractor’s fee and contingency	

For more accurate estimation of building costs, it is necessary to estimate the process area required based on the footprint of the equipment and the space required around the equipment for safe and efficient operation and maintenance. Then the building cost is estimated by multiplying the area of the various sections (e.g., process, laboratory, office) of a plant by an appropriate unit cost provided in Table 11.4. This table, which was developed by DPS Biometics (Framingham, MA), also provides information on air circulation rates for the various process areas, which determine the sizing and power requirements of HVAC systems.

Table 11.3 indicates a wide range in the equipment installation cost multipliers. Using multipliers that are specific to individual equipment items leads to the most accurate estimates. In general, equipment delivered mounted on skids has a lower installation cost.

For preliminary cost estimates, Table 11.3 clearly shows that the fixed capital investment of a plant is a multiple (usually 3 to 10 times) of its equipment purchase cost. The low end of the range applies to large scale facilities that produce biofuels and commodity biochemicals. The

high end applies to biopharmaceutical facilities.

TABLE 11.4

Building Cost Estimation (Year 2012 Prices) [2]

Space function	Unit cost ($/m^2)	Air circulation rates (volume changes/h)
Process areas[a]		
Class 100,000	3000–3750	20
Class 10,000	3750–5200	35–50
Class 1,000	6700–9000	100
Class 100	9000–12000	200–600
Mechanical room (utilities)	450–900	
Laboratory	1500–3000	
Office	750–900	

[a]The class number refers to the maximum number of particles 0.5 μm or larger per cubic foot.

The equipment purchase cost can be estimated from vendor quotations, published data, company data compiled from earlier projects, and by using process simulators that are equipped with appropriate costing capabilities. Vendor quotations are time-consuming to obtain and are, therefore, usually avoided for preliminary cost estimates. Instead, engineers tend to rely on the other three sources. Figures 11.7 to 11.10 provide equipment cost data for disk-stack centrifuges, membrane filtration systems, chromatography columns, and vertical agitated tanks that meet the specifications of the biopharmaceutical industry. The cost of the membrane filtration systems includes the cost of the skid, tank, pumps and automation hardware and software. The tanks are appropriate for buffer preparation. They include a low power agitator, but no heating/cooling jacket. The data represent average values from several vendors.

It should be noted that equipment purchase cost is a strong function of industrial application and plant location. The data of Figures 11.7 to 11.10 are applicable to biopharmaceutical facilities in developed countries. The cost of membrane filtration systems used in the food, bio-fuel and water purification industries is more than an order of magnitude lower compared to the biopharmaceutical industry. The much larger equipment scale and the less stringent equipment specifications relative to biopharmaceuticals are responsible for the large difference in cost. The same trend applies to the cost of chromatography columns, storage tanks, reactor vessels, and most other equipment items. A good source of cost data for equipment used in the bio-fuel and

biomaterial industries is available from the US Department of Energy [37]. Additional sources for bioprocessing equipment cost data are available in the literature [38, 39].

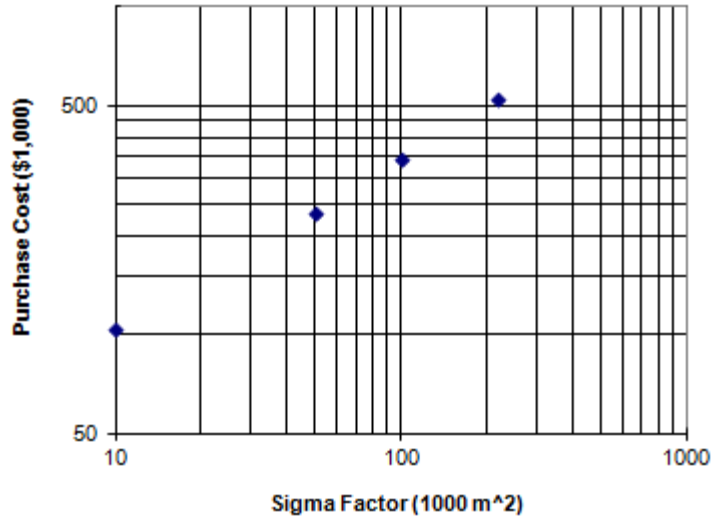

Figure 11.7 Purchase cost of disk-stack centrifuges vs. Σ Factor (2012 prices).

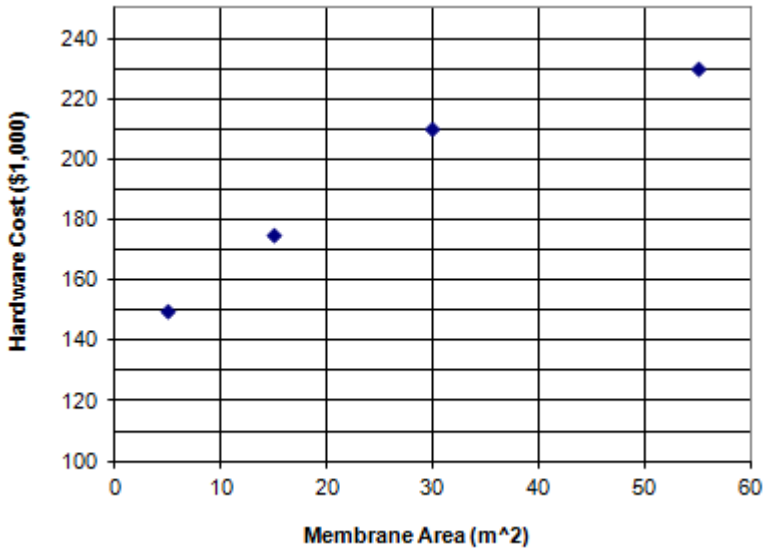

Figure 11.8 Purchase cost of membrane filtration systems (2012 prices)

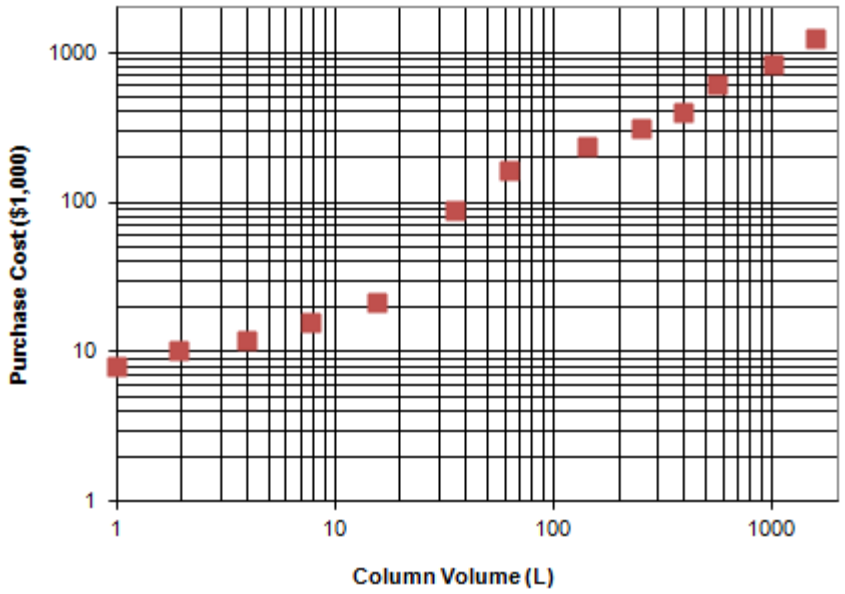

Figure 11.9 Purchase costs of chromatography columns made of acrylic tube and stainless steel bed supports (2012 prices).

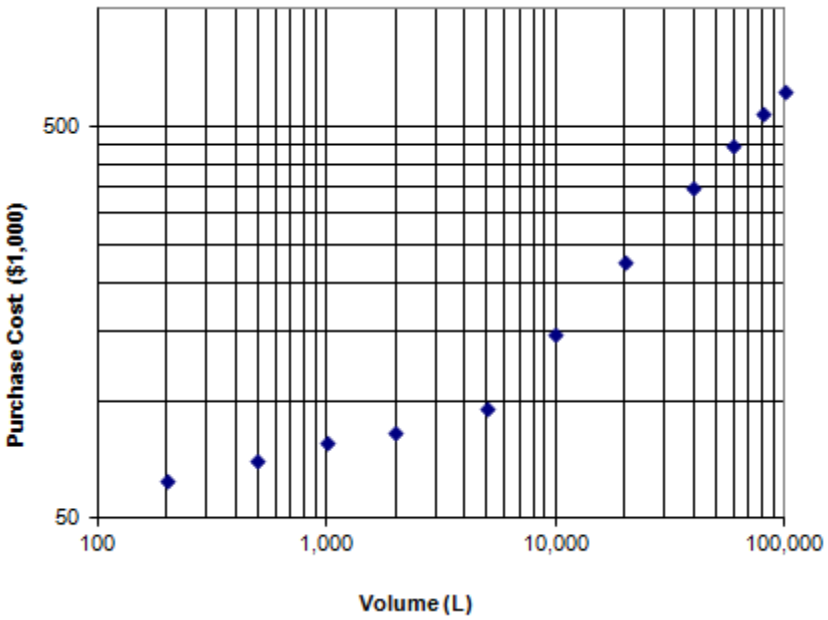

Figure 11.10 Purchase cost of agitated tanks made of stainless steel (2012 prices).

Often, cost data for one or two discrete equipment sizes are available, but the cost for a different size piece of equipment must be estimated. In such cases, the *scaling law* (expressed by the following equation) can be used:

$$\text{Cost}_2 = \text{cost}_1\left(\frac{\text{size}_2}{\text{size}_1}\right)^a \qquad (11.5.1)$$

The mathematical form of the scaling law explains why cost-versus-size data graphed on logarithmic coordinates tend to fall on a straight line. The value of the exponent *a* in Equation (11.5.1) ranges between 0.5 and 1.0, with an average value for vessels of around 0.6 (this explains why the scaling law is also known as the "0.6 rule"). According to this rule, when the size of a vessel doubles, its cost will increase by a factor of $(2/1)^{0.6}$, or approximately 52%. This result is often referred to as the *economy of scale*. In using the scaling law, it is important to make sure that the piece of equipment whose cost is being estimated has a size that does not exceed the maximum available size for that type of equipment.

The price of equipment changes with time owing to inflation and other market conditions. That change in price is captured by the Chemical Engineering Plant Cost Index (CE Index) that is published monthly by *Chemical Engineering* magazine. The index *I* is used to update equipment cost data according to the following equation:

$$\text{Cost}_2 = \text{cost}_1\frac{I_2}{I_1} \qquad (11.5.2)$$

Another factor that affects equipment purchase cost is the material of construction. For instance, a tank made of stainless steel costs approximately 2.5 to 3 times as much as a carbon steel tank of the same size. A tank made of titanium costs around 15 times the cost of a carbon steel tank of the same size. Other factors that affect equipment cost include the finishing of the metal surface and the instrumentation that is provided with the equipment.

Working Capital

Working capital accounts for cash that must be available for investments in on-going expenses and consumable materials. These expenses may include raw materials for 1 to 2 months, labor for 2 to 3 months, utilities for a month, waste treatment/disposal for a month, and other miscellaneous expenses. The required amount of working capital for a process is usually 10 to 20% of the DFC.

Start-up and Validation

Start-up and validation costs can also represent a significant capital investment for a biopharmaceutical plant. A value of 20 to 30% of DFC is quite common.

11.5.2 OPERATING COST ESTIMATION

The operating cost to run a biochemical plant is the sum of all on-going expenses including raw materials, labor, consumables, utilities, waste disposal and facility overhead. Dividing the annual operating cost by the annual production rate yields the unit production cost (e.g., in dollars per kilogram). The unit cost and selling prices of bioproducts are inversely proportional to market size (see Figure 1.1). Low molecular weight commodity biochemicals and biofuels that are produced in large quantities cost around $1 to $5/kg to make. Citric acid, whose production is analyzed later in this chapter, is a product of this type. Specialty biochemicals that are used as food supplements (e.g., vitamins) and flavoring agents have a manufacturing cost of $5 to $100/kg. The manufacturing cost of therapeutic proteins produced in large quantities is in the range of $1/g to $1000/g. Human serum albumin (HSA) which is extracted from blood plasma and has an annual production volume of more than 500 metric tons lies close to the low end. The manufacturing cost of therapeutic proteins with annual production volume ranging from a few hundreds of kilograms to a few metric tons is in the range of $50 to $1000/g. The insulin and monoclonal antibody processes analyzed later in this chapter represent products of this type. The manufacturing cost of interferons, erythropoietin (EPO) and other therapeutic proteins with very low annual production volume (from hundreds of grams to a few kilograms) is more than $10,000/g [40].

TABLE 11.5
Operating Cost Items and Ranges

Cost item	Type of cost	Range of values (% of total)
Raw materials	Direct	10–80
Labor	Direct	10–50
Consumables	Direct	1–50
Lab/QC/QA	Direct	1–50
Waste disposal	Direct	1–20
Utilities	Direct	1–30
Facility overhead	Indirect	10–70
Miscellaneous	Indirect	0–20

Table 11.5 displays the various types of operating cost, their direct or indirect nature, and ranges for their values relative to the total operating cost. Sometimes cost items are categorized as either fixed or variable. Fixed costs are those that are incurred regardless of the volume of product output. The clearest case of a fixed cost is depreciation, which is part of the equipment-dependent cost. The clearest case of a variable cost would be the cost of raw materials. Most

other costs have a fixed component and a variable component.

It is obvious from the wide range of values in Table 11.5 that industry averages cannot predict the operating cost of a process; a certain level of detailed calculations is required.

Raw Materials

The raw materials cost includes the cost of all fermentation media, recovery chemicals, and cleaning materials. For commodity bio-chemicals, such as ethanol, the cost of fermentation media is the main component. For high value products, the solutions used for product recovery and equipment cleaning can be a major part of the raw materials cost. Table 11.6 provides a list of commonly used raw materials in the biochemical industries. Note that the price of a raw material can vary widely depending on its required purity. This can be clearly seen in the case of water. Water for injection (WFI), for instance, costs 100 to 500 times as much as city water. Prices for a wide range of chemicals are available online at www.icis.com.

Labor

Labor is estimated based on the total number of operators, which in turn is calculated by summing up the operator requirements of the various operations as a function of time. As will become clear in the examples in Section 11.6, the labor requirement in a batch manufacturing facility varies with time. In a single-product facility, the number of operators in each shift must be based on maximum demand during that shift. In multiproduct facilities, each product line can employ a certain number of dedicated operators and rely on floating operators during periods of peak demand. In general, smaller facilities tend to utilize a larger number of operators per processing step because these plants are less automated. For instance, a small biotech company may utilize two or three operators to set up a fermentor, whereas in a large, highly automated fermentation facility a single operator may handle the setup of six different fermentors remotely from the control room. In general, a typical biotech company that deals with high value products will allocate at least one operator to each processing step (centrifugation, membrane filtration, chromatography, etc.) during its operation. The setup of a step may require multiple operators for a short period. The annual cost of an operator (including salary and benefits) varies widely around the globe. It is in the range of $4,000 to $10,000 in developing nations and can exceed $50,000 in developed countries [41].

TABLE 11.6
Common Bioprocessing Raw Materials (Year 2012 Prices)

Raw material	Comments	Price ($/kg)
C Source		
Glucose	Solution 70% w/v	0.30–0.40
Corn syrup	95% Dextrose equivalent	0.40–0.50
Molasses	50% Fermentable sugars	0.12–0.20
Soybean oil	Refined	1.10–1.30
Corn oil	Refined	1.30–1.40
Ethanol	USP tax free	0.80–0.90
Methanol	Gulf Coast	0.40–0.45
n-Alkanes		0.75–0.90
N Source		
Ammonia	Anhydrous, fertilizer grade	0.30–0.60
Soybean flour	44% protein	0.45–0.50
Cottonseed flour	62% protein	0.50–0.60
Casein	13.5% w/w total N	10.00–12.00
Ammonium sulfate	Technical	0.17–0.25
Ammonium nitrate	Fertilizer grade 33.5% N, bulk	0.20–0.30
Urea	46% N, agricultural grade	0.55–0.65
Yeast	Brewers, debittered	1.25–1.40
Whey	Dried, 4.5% w/w N	1.25–1.40
Salts		
KH_2PO_4	USP, granular	1.65–1.85
K_2SO_4	Granular, purified	2.80 --3.00
Na_2HPO_4		1.40–1.80
$MgSO_4 \cdot 7H_2O$		0.45–0.55
$ZnSO_4 \cdot 7H_2O$	Agricultural grade, powder	0.65–0.75
Other		
Process water		0.0001-0.001
RO water		0.005–0.01
Water for injection		0.02–0.5
H_3PO4 (85% w/w)	Food Grade	3.5 – 4.5
NaOH		0.2 – 0.5
HCl (37% w/w)		0.7 – 0.8
H_2SO_4 (98% w/w)		0.15–0.25

Consumables

Consumables are items that may be used up, fouled, or otherwise damaged during processing, such as membranes, chromatography resins, activated carbon, etc. These items must be periodically replaced. As the examples later in this chapter will illustrate, the high unit cost of chromatography resins and their frequent replacement can make them a major component of the

manufacturing cost. The unit cost of typical ion exchange and hydrophobic interaction chromatography resins used for the purification of proteins is in the range of $500 to $2,000 per liter of resin. The unit cost of protein-A affinity resins that are commonly used for the purification of monoclonal antibodies is in the range of $5,000 to $15,000 per liter of resin. The replacement frequency of such resins is in the range of 50 to 200 cycles of usage (the high-end resins have a longer useful life). In contrast, the unit cost of polymeric chromatography resins used for the purification of small bio-molecules (e.g., amino acids) is substantially lower (under $100 per liter of resin) and their life is longer (1000 to 2000 h of operation). Likewise, the unit cost of silica-based resins used for water demineralization is around $0.5/L and their life is in the range of 2000 to 6000 h of operation (the life strongly depends on the composition of the treated materials). Regarding membrane filtration operations, the unit cost of MF/UF membranes used in the biopharmaceutical industry (in the form of hollow-fiber cartridges or cassettes) is in the range of $300 to $800/$m^2$. Such membranes typically handle 10-50 filtration cycles before disposal. The unit cost of related membranes used in industrial biotechnology (e.g., for production of industrial enzymes) is considerably lower (under $200/m2) and the expected life is more than 2000 h of operation. The cost of membranes used for large scale water purification is under $50/$m^2$ and their useful life is at least 6000 h of operation. In general, ceramic membranes cost more than polymeric ones, but they last longer. The cost of disposable bags or containers, also known as single-use systems, is part of the consumables cost as well. Disposable bags have become popular in biopharmaceutical manufacturing because they eliminate the need for cleaning and sterilization in place [42]. Other advantages of single-use systems include increased processing flexibility and shorter validation, start-up and commercialization times. Table 11.7 provides information on disposable bags used for the preparation and storage of buffer solutions and fermentation media. Bags with mixing capability are required for solution preparation. Similar bags are used for inoculum preparation in rocking and stirred tank bioreactors. Bags for stirred tank bioreactors are available with working volumes of up to 2000 L. A number of biopharmaceuticals are produced exclusively in single-use systems. It should be noted that large disposables bags (larger than 50 L) utilize appropriate supporting skids. The SuperPro Designer databases provide cost information for such skids as well as for rocking and stirred tank bioreactor bags.

TABLE 11.7

Disposable Bags for Preparation and Storage of Solutions (Year 2012 prices)

Volume (L)	Bags for Storage ($)	Bags for Mixing ($)
50	310	600
100	340	690
200	360	820
500	460	930
1000	650	1180

Laboratory/QC/QA

Laboratory, QC, and QA activities include off-line analysis, quality control (QC), and quality assurance (QA) costs. Chemical and biochemical analysis and physical property characterization, from raw materials to final product, are a vital part of biochemical operations. The Laboratory/QC/QA cost is usually 10 to 20% of the operating labor cost. However, for certain biopharmaceuticals that require a large number of very expensive assays, this cost can be as high as the operating labor. For such cases, it is important to account for the number and frequency of the various assays in detail, since changes in lot size that can reduce the frequency of analysis can have a major impact on profit margins.

Waste Treatment and Disposal

The treatment of wastewater and the disposal of solid and hazardous materials is another important operating cost. The amount and composition of the various waste streams is derived from the material balances. Multiplying the amount of each waste stream by the appropriate unit cost yields the cost of treatment and disposal. Treatment of low biological oxygen demand (BOD) wastewater (<1000 mg/L) by a municipal wastewater treatment facility usually costs $0.2 to $0.5/$m^3$. This is not a major expense for most biotech facilities that deal with high value products. However, disposal of contaminated solvents (typically generated by chromatography steps) and other regulated compounds can become a major expense because the unit disposal cost can be more than $1/kg. Waste disposal may also become a problem if an unwanted by-product is generated as part of the recovery chemistry of a process (see the citric acid example, Section 11.6.1). Disposal of single-use systems via incineration costs $100 to $200 per metric ton of material.

Utilities

Utilities costs include the cost of heating and cooling agents as well as electricity. The amounts are calculated as part of the material and energy balances. Aerobic fermentors are major consumers of electricity, but downstream processing equipment generally does not consume much electricity. In terms of unit cost, electricity costs $0.05 to $0.15/kWh. The cost of heat removal using cooling water is in the range of $0.002 to $0.01 per 1000 kcal of heat removed. The cost of cooling using chilled water and refrigerants is in the range of $0.05 to $0.1 per 1000 kcal of heat removed. The cost of producing steam for use as a heating medium is around $5 to $15/1000 kg depending on pressure (low, medium, high), type of fuel used for its generation and scale of production. The cost of clean steam (generated utilizing highly purified water) is around $50 to $100/1000 kg (depending on the scale of production and level of water purity). Clean steam is used in biopharmaceutical facilities for sterilizing equipment as part of equipment cleaning (e.g., "steam in place" or SIP operations). Note that manufacturers often classify purified water used for buffer preparation and equipment cleaning as a utility and not as a raw material, thus increasing the cost contribution of utilities. The insulin example, presented later in this chapter, describes a methodology for the systematic sizing of systems that supply purified water.

Facility Overhead

Facility overhead costs account for the depreciation of the fixed capital investment, maintenance costs for equipment, insurance, local (property) taxes, and possibly other overhead-type expenses. For preliminary cost estimates, the entire fixed capital investment is usually depreciated linearly over a 10-year period. In the real world, the U.S. government allows corporations to depreciate equipment in 5 to 7 years and buildings in 25 to 30 years. The value of land cannot be depreciated. The annual maintenance cost can be estimated as a percentage of the equipment's purchase cost (usually 10%) or as a percentage of the overall fixed capital investment (usually 3-5%). Insurance rates depend to a considerable extent upon the maintenance of a safe plant in good repair condition. A value for insurance in the range of 0.5 to 1% of DFC is appropriate for most bioprocessing facilities. The processing of flammable, explosive, or highly toxic materials usually results in higher insurance rates. The local (property) tax is usually 2 to 5% of DFC. The factory expense represents overhead cost incurred by the

operation of non-process-oriented facilities and organizations, such as accounting, payroll, fire protection, security, and cafeteria. A value of 5 to 10% of DFC is appropriate for these costs.

Miscellaneous

Included in miscellaneous costs are ongoing R&D, process validation, and other overhead-type expenses that can be ignored in preliminary cost estimates. Other general expenses of a corporation include royalties, advertising, and selling. If any part of the process or any equipment used in the process is covered by a patent not assigned to the corporation undertaking the new project, permission to use the technology covered by the patent must be negotiated, and some form of royalty or license fee is usually required. Advertising and selling covers expenses associated with the activities of the marketing and sales departments.

11.5.3 PROFITABILITY ANALYSIS

Estimates of capital investment, operating cost, and revenues of a project provide the information needed to assess its profitability and attractiveness from an investment point of view. There are various measures for assessing profitability. The simplest ones include gross margin, return on investment (ROI), and payback time, and they are calculated by using the following equations:

$$\text{Gross margin} = \frac{\text{gross profit}}{\text{revenues}} \tag{11.5.3}$$

$$\text{Return on investment (ROI)} = \frac{\text{net profit per year}}{\text{total investment}} \times 100\% \tag{11.5.4}$$

$$\text{Payback time (years)} = \frac{\text{total investment}}{\text{net profit per year}} \tag{11.5.5}$$

where gross profit is equal to annual revenues minus the annual operating cost, and net profit is equal to gross profit minus income taxes plus depreciation. All variables are averaged over the lifetime of a project.

Other measures that are more involved, such as the net present value (NPV) and internal rate of return (IRR), consider the cash flows of a project over its evaluation life and the time-value of money. Detailed definitions for NPV and IRR can be found in the literature [31, 36]. The examples presented next demonstrate how these measures facilitate the decision-making process.

11.6 Illustrative Examples

In this section, SuperPro Designer is used to illustrate the analysis and evaluation of the production of three biological products. The first example analyzes the production of citric acid, a commodity organic acid heavily used in the beverage industry. The second deals with the

bacterial production of recombinant human insulin, the first commercial product of modern biotechnology. The third example focuses on the production of monoclonal antibodies (MAbs) from mammalian cells cultured in stirred tank bioreactors. The generation of the flowsheets for the production of all three products was based on information available in the patent and technical literature combined with our engineering judgment and experience with other biological products. We use these examples to draw general conclusions on the manufacturing cost of biological products. The computer files for these examples are available as part of the evaluation version of SuperPro Designer at the website www.intelligen.com. Additional examples and pertinent publications are available at www.intelligen.com/literature.

11.6.1 CITRIC ACID PRODUCTION

A number of organic acids are produced via fermentation. Of these, citric acid is produced in the largest amount (>1,800,000 metric tons per year). Citric acid is marketed as citric acid monohydrate or as anhydrous citric acid. The majority of citric acid (>60%) is used in the food and beverage industries to preserve and enhance flavor. In the chemical industries (which represent 25–30% of total utilization), the uses of citric acid include the treatment of textiles, softening of water, and manufacturing of paper. In the pharmaceutical industry (10% of total utilization), iron citrate is used as a source of iron, and citric acid is used as a preservative for stored blood, tablets, and ointments, and in cosmetic preparations [43]. Citric acid is increasingly being used in the detergent industry as a replacement for polyphosphates.

Citric acid was first recovered in 1869 in England from calcium citrate, which was obtained from lemon juice. Its production by filamentous fungi has been known since 1893. The first production via surface culture fermentation was initiated in 1923. Production using stirred tank fermentors began in the 1930’s, and presently this is the preferred method for large-scale manufacturing. The plant considered in this example produces around 18,000 metric tons of crystal citric acid per year, which represents approximately 1% of the current world demand.

Process Description

Upstream Section The entire flowsheet is shown in Figure 11.11. Molasses, the carbon source of fermentation, is diluted with water from about 50% fermentable sugars content to 20% in a blending tank (V-101). Suspended particulate material is then removed by filtration (PFF-101). Metal ions, particularly iron, are subsequently removed by an ion exchange chromatography column (C-101) and the purified raw material solution is then heat-sterilized (ST-101).

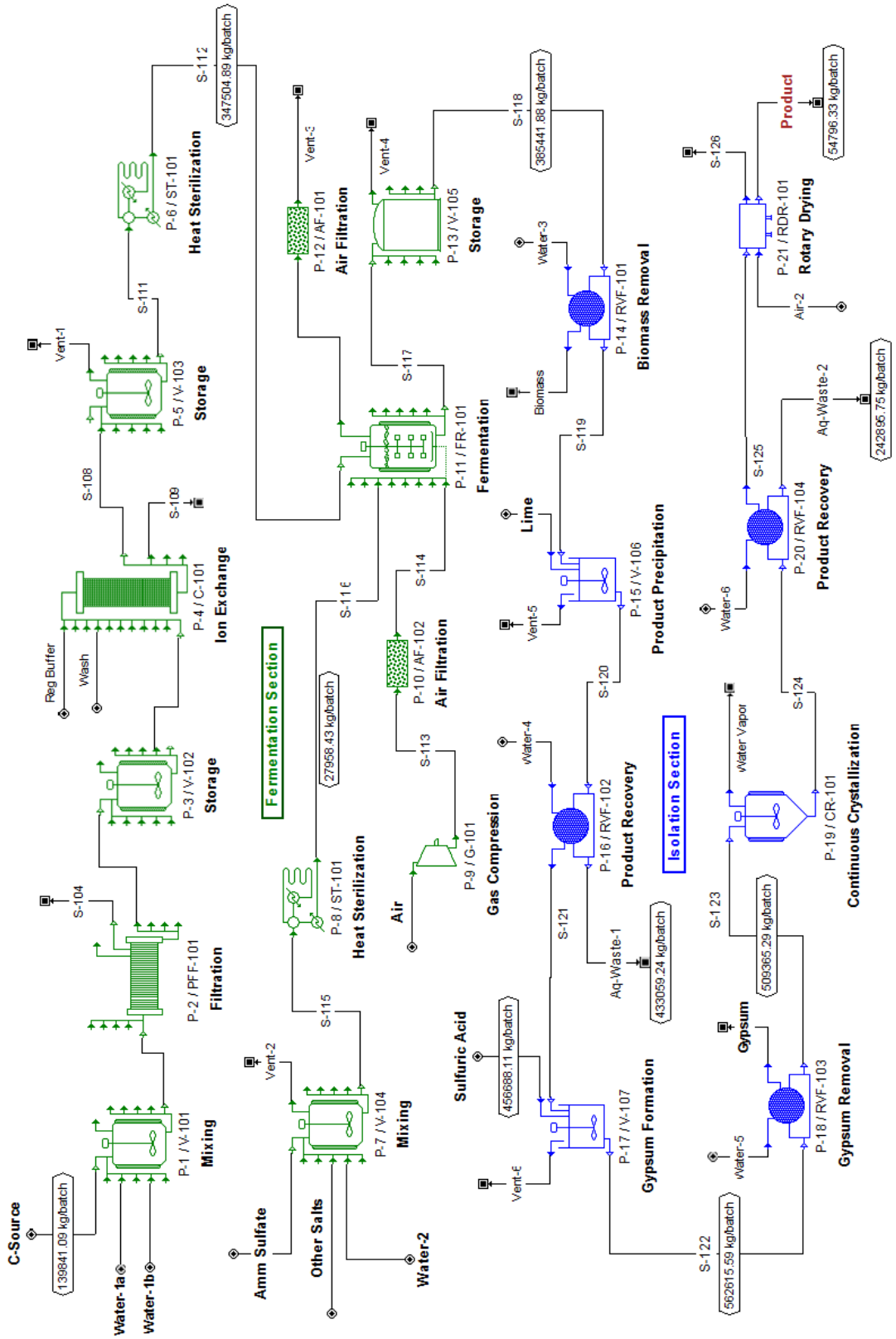

Figure 11.11 Citric acid production flowsheet.

Nutrients (i.e., sources of ammonium, potassium, phosphorus, magnesium, copper, and zinc) are dissolved in water (V-104) and heat-sterilized (ST-101). The fermentation cycle is 7 days, and the production is handled by seven fermentors that operate in staggered mode. Since the plant operates around the clock, one fermentation batch is initiated daily and another one is completed daily. Each fermentor has a vessel volume of 350 m^3 and generates broth of around 315 m^3. A three-step seed fermentor train (not shown in the flowsheet) supplies inoculum to each production fermentor (FR-101). A pure culture of the mold *Aspergillus niger* is used to inoculate the smallest seed fermentor. When optimum growth of mycelium is reached, the contents of the seed fermentor are transferred to the next stage fermentor, which is approximately 10 times larger. Similarly, this larger seed fermentor inoculates the production fermentor with about 10% volume of actively growing mycelium broth. Air is supplied by a compressor (G-101) at a rate that gradually increases from 0.15 VVM (volume of air per volume of liquid per minute) to 1.0 VVM. Cooling water removes the heat produced by the exothermic process (2990 kcal per kg of citric acid formed) and maintains the temperature at 28°C. The fermented broth is discharged into the holding tank (V-105), which acts as a buffer tank between the batch upstream section and the continuous downstream section.

Downstream Section Purification starts with the removal of biomass by a rotary vacuum filter (RVF-101). The clarified fermentation liquor flows to an agitated reactor vessel (V-106) where approximately 1 part of hydrated lime, $Ca(OH)_2$, for every 2 parts of liquor is slowly added in order to precipitate calcium citrate. The lime solution must be very low in magnesium content in order to minimize losses due to generation of relatively soluble magnesium citrate. Calcium citrate is separated by a second rotary vacuum filter (RVF-102) and the citrate-free filtrate (Aq-Waste-1) is sent to a wastewater collection tank. The calcium citrate cake is sent to another agitated reactor vessel (V-107), where it is acidified with dilute sulfuric acid to form a precipitate of calcium sulfate (gypsum). A third filter (RVF-103) removes the precipitated gypsum and yields an impure citric acid solution in the filtrate. Careful control of the pH and temperature of each precipitation step is important for maximizing the yield of citric acid. The resulting solution is concentrated and crystallized using a continuous evaporator/crystallizer (CR-101). The crystals formed are separated by rotary vacuum filtration (RVF-104) and dried in a rotary dryer (RDR-101). If the final product is required in high purity, treatment with activated carbon may

precede crystallization to remove colorants. In addition, ion exchange is sometimes used to remove metal ions and other ionic species.

Process Scheduling

Figure 11.12 displays the equipment occupancy chart for 14 consecutive batches. The process batch time is approximately 200 h (or 8.3 days). This is the time elapsed from the preparation of raw materials to the final product of a single batch (excluding the time required for inoculum preparation). The duration of each fermentation batch is 160 h (6.7 days). The availability of 7 production fermentors operating in staggered mode (out of phase) enable the plant to initiate a new batch every 24 h. The upstream portion of the process (i.e., raw material preparation and fermentation) operates in batch mode. The downstream section (product recovery and purification) operates continuously. The continuous units of the purification train are displayed in Figure 11.12 with aligned blocks that operate back-to-back (lower portion of the chart).

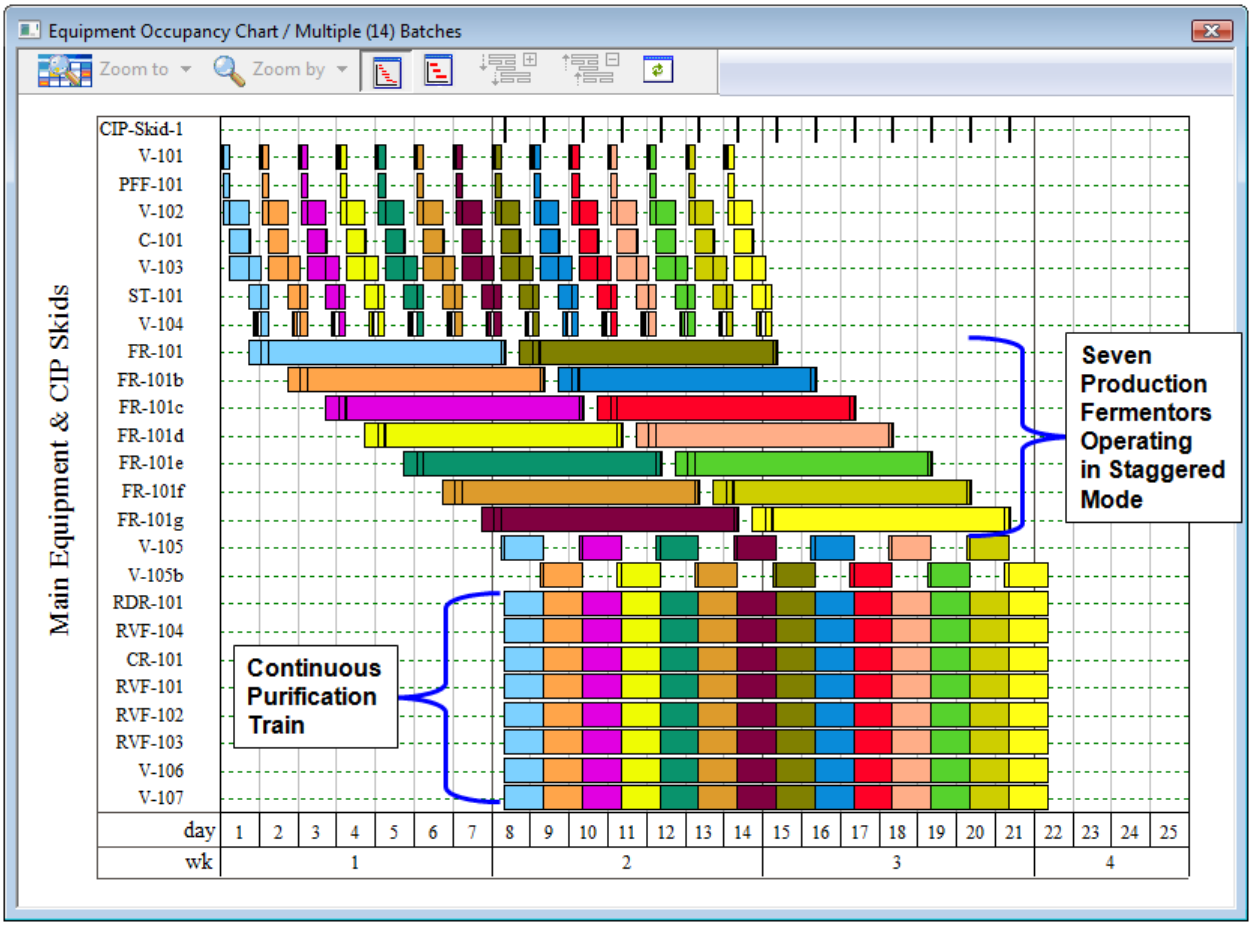

Figure 11.12 Equipment occupancy chart for 14 consecutive batches of the citric acid process.

Material Balances

Table 11.8 provides a summary of the overall material balances (expressed as input-output component balance). "CA crystal" stands for crystalline citric acid and represents the final product. Glucose represents the fermentable carbohydrates in molasses (50% w/w). Note the large amounts of $Ca(OH)_2$ and sulfuric acid consumed, and gypsum (calcium sulfate) generated. The quantities of these compounds depend on the chemistry of the purification process and cannot be reduced without changing the recovery technology. Since this gypsum is contaminated with biomass, it has little or no commercial value. A disposal cost of $50/MT (metric ton) was assumed in this example. The large amount of wastewater is also worth noting.

TABLE 11.8
Overall Material Balances for Citric Acid (CA) Production (kg/year)

Component	In	Out	(Out − in)
Ammonium sulfate	278,000	26,000	−252,000
Biomass	0	2,014,000	2,014,000
CA crystal	0	18,250,000	18,250,000
$Ca(OH)_2$	11,717,000	558,000	−11,159,000
Calcium citrate	0	623,000	623,000
CO_2	0	3,861,000	3,861,000
Citric acid	0	657,000	657,000
Glucose	22,934,000	275,000	−22,659,000
Gypsum	0	19,972,000	19,972,000
Impurities	459,000	459,000	0
Nutrients	1,894,000	383,000	−1,511,000
Oxygen	65,171,000	57,994,000	−7,177,000
NaOH	185,000	185,000	0
Sulfuric acid	14,979,000	576,000	−14,403,000
Water	308,879,000	320,663,000	11,784,000
Total	426,496,000	426,496,000	0

Economic Evaluation

Table 11.9 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 is around $10.4 million. Note that approximately 30% of the equipment cost is associated with the seven production fermentors. The fermentors are made of stainless steel to minimize leaching of heavy metals that affect product formation. The final item, "cost of unlisted equipment," accounts for the cost of the seed fermentors, pumps and other secondary equipment that is not considered explicitly. Table 11.10 displays the various items of the direct fixed capital (DFC) investment.

The total DFC for a plant of this capacity is around $43.6 million or approximately 4.2 times the total equipment cost.

TABLE 11.9

Equipment Specification and Purchase Costs for Citric Acid Production (Year 2012 Prices)

Quantity	Name	Description	Unit cost	Cost
1	AF-101	Air Filter Rated Throughput = 7.7 m3/s	100,000	100,000
1	AF-102	Air Filter Rated Throughput = 3.0 m3/s	37,000	37,000
1	C-101	Chromatography Column Column Volume = 4.66 m3	150,000	150,000
1	CR-101	Crystallizer Vessel Volume = 130 m3	542,000	542,000
7	FR-101	Fermentor Vessel Volume = 350 m3	436,000	3,052,000
1	G-101	Centrifugal Compressor Compressor Power = 1,430 kW	1,560,000	1,560,000
1	PFF-101	Plate & Frame Filter Filter Area = 335 m2	145,000	145,000
1	RDR-101	Rotary Dryer Drying Area = 85 m2	475,000	475,000
1	RVF-101	Rotary Vacuum Filter Filter Area = 46 m2	154,000	154,000
1	RVF-102	Rotary Vacuum Filter Filter Area = 83 m2	214,000	214,000
1	RVF-103	Rotary Vacuum Filter Filter Area = 71 m2	195,000	195,000
1	RVF-104	Rotary Vacuum Filter Filter Area = 35 m2	137,000	137,000
1	ST-101	Sterilizer Rated Throughput = 34 m3/h	308,000	308,000
1	V-101	Blending Tank Vessel Volume = 300 m3	503,000	503,000
1	V-102	Blending Tank Vessel Volume = 300 m3	503,000	503,000
1	V-103	Blending Tank Vessel Volume = 300 m3	503,000	503,000
1	V-104	Blending Tank Vessel Volume = 35 m3	139,000	139,000
2	V-105	Flat Bottom Tank Vessel Volume = 350 m3	198,000	396,000
1	V-106	Neutralizer Vessel Volume = 42 m3	126,000	126,000
1	V-107	Neutralizer Vessel Volume = 15 m3	94,000	94,000
		Unlisted Equipment		1,037,000
		Total		**10,370,000**

TABLE 11.10
Fixed Capital Estimate Summary for Citric Acid Production (Year 2012 Prices)

Total plant direct cost (TPDC)		
Equipment purchase cost	10,370,000	
Installation	3,726,000	
Process piping	3,111,000	
Instrumentation	2,074,000	
Insulation	311,000	
Electricals	1,037,000	
Buildings	2,074,000	
Yard improvement	1,556,000	
Auxiliary facilities	1,037,000	
TPDC		25,295,000
Total plant indirect cost (TPIC)		
Engineering	5,059,000	
Construction	7,589,000	
TPIC		12,648,000
Total plant cost (TPC = TPDC + TPIC)		37,943,000
Contractor's fee	1,897,000	
Contingency	3,794,000	
Direct fixed capital (DFC) TPC + contractor's fee and contingency		43,634,000

Table 11.11 provides a summary of the operating costs. The raw materials cost is the most important, accounting for 40.9% of the overall operating cost. This is quite common for commodity bio-chemicals. Molasses is the most expensive raw material, accounting for 67% of the raw materials cost. The purification chemicals, sulfuric acid and calcium hydroxide, account for 18.7% and 9.3% of the overall raw materials cost, respectively. The following prices were assumed: \$0.15/kg of molasses, \$0.013/kg of 10% w/w H_2SO_4 solution, \$0.08/kg of $Ca(OH)_2$, and \$0.1/m^3 of process water. The facility-dependent cost is the second most important, accounting for 28.7% of the overall cost. Depreciation of the fixed capital investment and maintenance of the facility are the main contributors to this cost. Utilities are the third largest expense, accounting for 14.8% of the overall cost. Electricity and cooling water utilized by the fermentors are the main contributors to this cost. Labor lies in the fourth position, and the environmental cost (waste treatment/disposal) is fifth. Disposal unit costs of \$1/m^3 and \$50/MT (metric ton) were assumed for liquid and solid (gypsum) waste streams, respectively. The

disposal of gypsum accounts for 85% of the overall environmental cost. The overall unit production cost is approximately $1.4/kg, which is roughly equal to the current (early 2012) selling price of citric acid [44]. This can be explained by noting the excess citric acid production capacity around the world (which keeps profit margins low), and the fact that most operating citric acid plants are rather old and partially depreciated. If depreciation is ignored, the facility-dependent cost is reduced by more than 80% and the overall unit cost drops to around $1/kg.

TABLE 11.11
Operating Cost Summary for Citric Acid Production (Year 2012 Prices)

Cost item	($/kg citric acid crystals)	Annual cost ($/year)	Proportion of total (%)
Raw materials	0.57	10,310,000	40.92
Facility-Dependent	0.40	7,223,000	28.67
Labor	0.12	2,102,000	8.34
Consumables	0.00	15,000	0.06
Lab/QC/QA	0.01	210,000	0.83
Waste treatment and disposal	0.09	1,611,000	6.39
Utilities	0.21	3,724,000	14.78
Total	1.40	22,195,000	100.00

Based on the preliminary evaluation of this project idea, one should not recommend investing in new citric acid production capacity unless there is a combination of favorable conditions. Obviously, availability of inexpensive equipment (e.g., by acquiring an existing facility) and raw materials (e.g., by locating the plant near a source of low cost molasses) are the most important factors. Development or adoption of a superior technology may also change the attractiveness of citric acid production. Such a technology is actually available; it utilizes extraction for citric acid recovery [45]. Recovery by extraction eliminates the consumption of $Ca(OH)_2$ and H_2SO_4 and the generation of the unwanted $CaSO_4$. Butanol has been used as an extractant, as has tributyl phosphate. Ion pair extraction by means of secondary or tertiary amines dissolved in a water-immiscible solvent (e.g., octyl alcohol) provides an alternative route. With recent developments in electrodialysis membranes, the use of this technique to recover citric acid directly from the fermentation broth may become an attractive alternative [46].

11.6.2 HUMAN INSULIN PRODUCTION

Introduction

Insulin facilitates the metabolism of carbohydrates and is essential for the supply of energy to the

cells of the body. Impaired insulin production leads to the disease diabetes mellitus, which is the third largest cause of death in industrialized countries, after cardiovascular diseases and cancer [47].

Human insulin is a polypeptide consisting of 51 amino acids arranged in two chains: chain A with 21 amino acids, and chain B consisting of 30 amino acids. The A and B chains are connected by two disulfide bonds. Human insulin has a molecular weight of 5808 and an isoelectric point of 5.4. Human insulin can be produced by four different methods:

- Extraction from human pancreas
- Chemical synthesis via individual amino acids
- Conversion of pork insulin, or "semisynthesis"
- Fermentation of genetically engineered microorganisms

Extraction from the human pancreas cannot be practiced because the availability of raw material is so limited. Total synthesis, while technically feasible, is not economically viable because the yield is very low. Production based on pork insulin, also known as "semisynthesis," transforms the porcine insulin molecule into an exact replica of the human insulin molecule by substituting a single amino acid, threonine, for alanine in the G-30 position. This technology has been developed and implemented by Novo Nordisk A/S (Denmark). However, this option is also quite expensive because it requires the collection and processing of large amounts of porcine pancreases. In addition, the supply is limited by the availability of porcine pancreas.

At least three alternative technologies have been developed for producing human insulin based on fermentation and utilizing recombinant DNA technology [48].

Two-Chain Method The first successful technique of biosynthetic human insulin (BHI) production based on recombinant DNA technology was the two-chain method. This technique was developed by Genentech, Inc. (South San Francisco) and scaled up by Eli Lilly and Company (Indianapolis). Each insulin chain is produced as a β-galactosidase fusion protein in *Escherichia coli*, forming inclusion bodies. The two peptide chains are recovered from the inclusion bodies (IBs), purified, and combined to yield human insulin. Later, the β-galactosidase operon was replaced with the tryptophan (Trp) operon, resulting in a substantial yield increase.

Proinsulin Method The so-called intracellular method of making proinsulin eliminates the need for the separate fermentation and purification trains required by the two-chain method. Intact proinsulin is produced instead. The proinsulin route has been commercialized by Eli Lilly

[49]. Figure 11.13 shows the key transformation steps. The *E. coli* cells overproduce Trp-LE´-Met-proinsulin (Trp-LE´-Met is a 121 amino acid peptide signal sequence; proinsulin, with 82 amino acids, is a precursor to insulin) in the form of inclusion bodies, which are recovered and solubilized. Proinsulin is released by cleaving the methionine linker using CNBr. The proinsulin chain is subjected to a folding process to allow intermolecular disulfide bonds to form; and the C peptide, which connects the A and B chains in proinsulin, is then cleaved with enzymes to yield human insulin. A number of chromatography and membrane filtration steps are required to purify the product.

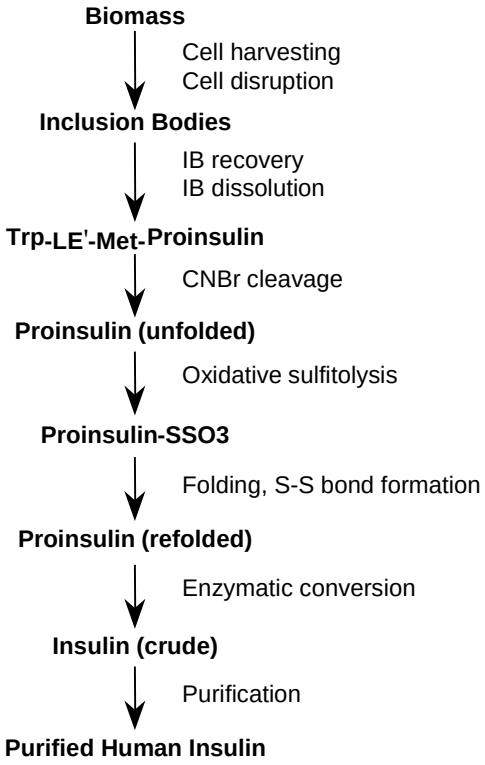

Figure 11-13 Human insulin from proinsulin fusion protein.

A second (extracellular) method of producing proinsulin was developed by Novo Nordisk A/S. It is based on yeast cells that secrete insulin as a single-chain insulin precursor [47]. Secretion simplifies product isolation and purification. The precursor contains the correct disulfide bridges and is therefore identical to those of insulin. It is converted to human insulin by transpeptidation in organic solvent in the presence of a threonine ester and trypsin followed by de-esterification. Another advantage of the secreted proinsulin technology is that by employing a continuous bioreactor–cell separator loop, it is possible to reuse the cells.

In this example, we analyze a process based on the intracellular proinsulin method.

Market Analysis and Design Basis

The annual world demand for insulin and insulin analogs was over 100,000 kg in 2010 and was growing at an annual rate of around 20% [50, 51, and 52]. The plant analyzed in this example has a capacity of around 1800 kg of purified biosynthetic human insulin (BHI) per year. This is a relatively large plant for producing polypeptide-based biopharmaceuticals. The plant operates around the clock, 330 days a year. A new batch is initiated every 48 h, resulting in 160 batches per year. The fermentation broth volume per batch is approximately 37.5 m^3.

Process Description

The entire flowsheet for the production of BHI is shown in Figure 11.14. It is divided into four sections: Fermentation, Primary Recovery, Reactions, and Final Purification. *Note:* A "section" in SuperPro is simply a set of unit procedures (processing steps). If you open the file "insulin.spf" in SuperPro, you will see that all of the unit procedures within a given section have their own distinctive color (blue, green, purple, and black for Fermentation, Primary Recovery, Reactions, and Final Purification, respectively).

Fermentation Section Fermentation media is prepared in a stainless steel tank (BT-101) and sterilized in a continuous heat sterilizer (ST-101). The axial compressor (G-101) and the absolute filter (AF-101) provide sterile air and ammonia to the fermentor at an average rate of 0.5 VVM. A two-step seed fermentor train (not shown in the flowsheet) is used to inoculate the 50 m^3 production fermentor (FR-101) with transformed *E. coli* cells. These cells are used to produce the Trp-LE´-Met-proinsulin precursor of insulin, which is retained in the cellular biomass. The fermentation time in the production fermentor is about 18 h, and the fermentation temperature is 37°C. The final concentration of *E. coli* in the production fermentor is about 30 g/L (dry cell weight). The Trp operon is turned on when the *E. coli* fermentation runs out of tryptophan. The chimeric protein Trp-LE´-Met-proinsulin accumulates intracellularly as insoluble aggregates (inclusion bodies), and this decreases the rate at which the protein is degraded by proteolytic enzymes. In the base case, it was assumed that the inclusion bodies (IBs) constitute 20% of total dry cell mass. At the end of fermentation, the broth is cooled down to 10°C to minimize cell lysis. After completing each processing step in the fermentation section (and subsequent sections), the equipment is washed to prepare for the next batch of product.

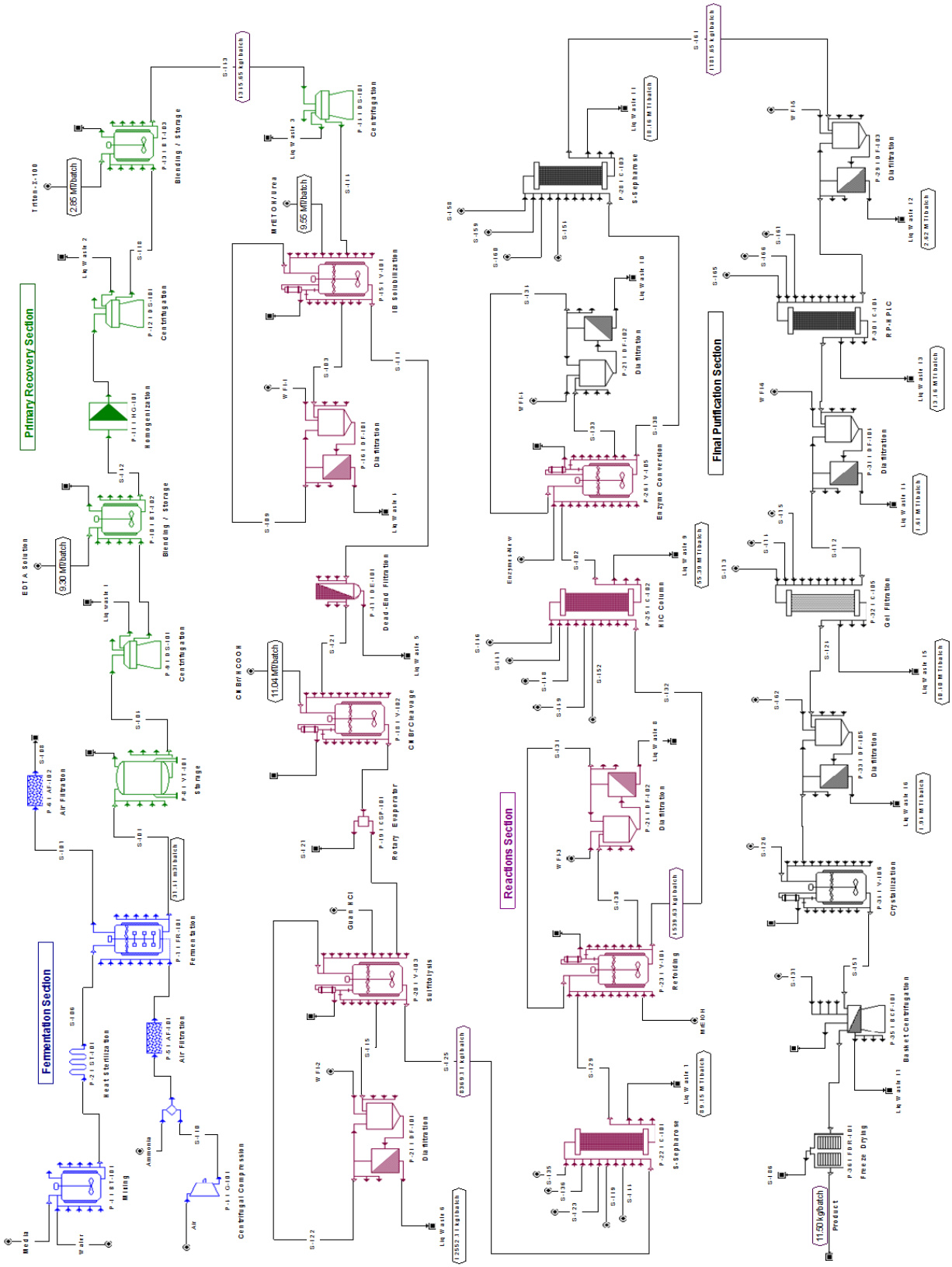

Figure 11.14 Insulin production flowsheet.

Primary Recovery Section After the end of fermentation, the broth is transferred into a surge tank (VT-101), which isolates the upstream section from the downstream section of the plant. Three disk-stack centrifuges (DS-101) operating in parallel are used for cell harvesting. Note that a single unit procedure icon in the SuperPro model may represent multiple equipment items operating in parallel (to see the number of equipment items a particular icon represents, right-click on the icon, go to Equipment Data, and look at the "Number of Units" field on the Equipment tab). During centrifugation, the broth is concentrated from 37,000 L to 9,157 L, and most of the extracellular impurities are removed. The cell recovery yield is 98%. The cell sludge is diluted with an equal volume of buffer solution (buffer composition: 96.4% w/w water for injection (WFI), 0.7% EDTA, and 2.9% Tris-base) in a blending tank (BT-102). The buffer facilitates the separation of the cell debris particles from inclusion bodies. Next, a high pressure homogenizer (HG-101) is used to break the cells and release the inclusion bodies. The exit temperature is maintained at around 10°C. The same centrifuges as before (DS-101) are then used for inclusion body recovery (P-12). The reuse of these centrifuges can be seen by noting that procedures P-9 and P-12 have the same equipment name, DS-101. The IBs are recovered in the heavy phase (with a yield of 98%) while most of the cell debris particles remain in the light phase. This is possible because the density (1.3 g/cm^3) and size (diameter about 1 μm) of the IBs are significantly greater than that of the cell debris particles. The IB sludge, which contains approximately 20% solids w/w, is washed with WFI containing 0.66% w/w Triton-X100 detergent (the volume of solution is twice the volume of inclusion body sludge) and re-centrifuged (P-14) using the same centrifuges as before (DS-101). The detergent solution facilitates purification (dissociation of debris and soluble proteins from inclusion bodies). The exit temperature is maintained at 10°C. The slurry volume at the end of the primary recovery section is around 1440 L.

Reactions Section *Inclusion Body Solubilization*. The inclusion body suspension is transferred to a glass-lined reaction tank (V-101) and is mixed with urea and 2-mercaptoethanol to final concentrations of 300 g/L (5 M) and 40 g/L, respectively. Urea is a chaotropic agent that dissolves the denatured protein in the inclusion bodies, and 2-mercaptoethanol is a reductant that reduces disulfide bonds. A reaction time of 8 h is required to reach a solubilization yield of 95%. The inclusion bodies are composed of 80% w/w Trp-LE´-Met-proinsulin, with the remainder

being other (contaminant) proteins. At the end of the solubilization reaction, a diafiltration unit (DF-101) is used to replace urea and 2-mercaptoethanol with WFI and to concentrate the solution. This operation is performed in 6 h with a recovery yield of 98%. All remaining fine particles (biomass, debris, and inclusion bodies) are removed by means of a polishing dead-end filter (DE-101). This polishing filter protects the chromatographic units that are used further downstream. The solution volume at this point is around 2200 L.

CNBr Cleavage. The chimeric protein is cleaved with CNBr (cyanogen bromide) into the signal sequence Trp-LE´-Met, which contains 121 amino acids, and the denatured proinsulin (82 amino acids) in a glass-lined reactor (V-101). The reaction is carried out in a 70% formic acid solution containing 30-fold molar excess CNBr (stoichiometrically, one mole of CNBr is required per mole of Trp-LE´-Met-proinsulin). The reaction takes 12 h at 20°C and reaches a yield of 95%. The mass of the released pro-insulin is approximately 30% of the mass of Trp-LE´-Met-proinsulin. A small amount of cyanide gas is formed as a by-product of the cleavage reaction. Detailed information on CNBr cleavage is available in the patent literature [53]. The formic acid, unreacted CNBr, and generated cyanide gas are removed by applying vacuum and raising the temperature to around 35°C (the boiling point of CNBr). This operation is carried out in a rotary vacuum evaporator (CSP-101) and takes 1 h. Since cyanide gas is toxic, all air exhausted from the vessels is scrubbed with a solution of hypochlorite, which is prepared and maintained in situ [49].

Sulfitolysis. Sulfitolysis of the denatured proinsulin takes place in a glass-lined reactor (V-103) under alkaline conditions (pH 9–11). This operation is designed to unfold pro-insulin, break any disulfide bonds, and add SO_3 moieties to all sulfur residues on the cysteines. The product of interest is human proinsulin$(S—SO_3—)_6$ (protein–S–sulfonate). The sulfitolysis step is necessary for two reasons: (1) the proinsulin probably is not folded in the correct configuration when expressed in *E. coli* as part of a fusion protein, and (2) the cyanogen bromide treatment tends to break existing disulfide bonds. The final sulfitolysis mixture contains 50% w/w guanidine HCl (6 M), 0.35% ammonium bicarbonate (NH_4HCO_3), 3% Na_2SO_3, and 1.5% $Na_2S_4O_6$ [54]. A reaction time of 12 h is required to reach a yield of 95%. The presence of the denaturing reagent (guanidine HCl) prevents re-folding and cross-folding of the same protein molecule onto itself or two separate protein molecules onto each other. Urea may also be used as a denaturing reagent. Upon completion of the sulfitolysis reaction, the sulfitolysis solution is exchanged with WFI to a

final guanidine HCl concentration of 20% w/w. This procedure, P-21, utilizes the DF-101 diafilter that also handles buffer exchange after IB solubilization. The human proinsulin(S—SO_3—)$_6$ is then chromatographically purified by means of three ion exchange columns (C-101) operating in parallel and each running four cycles per batch. Each column has a diameter of 140 cm and a bed height of 25 cm. A cation exchange resin is used (SP Sepharose Fast Flow from Amersham-Pharmacia Biotech) operating at pH 4.0. The eluant solution contains: 69.5% w/w WFI, 29% urea, and 1.5% NaCl. Urea, a denaturing agent, is used to prevent incorrect refolding and cross-folding of proinsulin(S—SO_3—)$_6$. The following operating assumptions were made: (1) the column is equilibrated for 30 min prior to loading, (2) the total resin binding capacity is 20 mg/mL, (3) the eluant volume is equal to 5 column volumes (CVs), (4) the total volume of the solutions for column wash, regeneration, and storage is 15 CVs, and (5) the protein of interest is recovered in 1.5 CVs of eluant buffer with a recovery yield of 90%.

Refolding. This operation catalyzes the removal of the SO_3 moiety and then allows disulfide bond formation and correct refolding of the proinsulin to its native form. It takes place in a reaction tank (V-104). This process step involves treatment with mercaptoethanol (MrEtOH), a reductant that facilitates the disulfide interchange reaction. It is added at a ratio of 1.5 mol of mercaptoethanol to 1 mol of SO_3. Dilution to a proinsulin(S-SO_3-)$_6$ concentration of less than 1 g/L is required to prevent cross-folding of proinsulin molecules. The reaction is carried out at 8°C for 12 h and reaches a yield of 85%. After completion of the refolding step, the refolding reagents are replaced with WFI and the protein solution is concentrated using a diafiltration unit (DF-102), which has a product recovery yield of 95% (5% of the protein denatures). The volume of the solution at this point is around 4500 L. Next, the human proinsulin is chromatographically purified in a hydrophobic interaction chromatography (HIC) column (C-102). The following operating assumptions were made: (1) the column is equilibrated for 30 min prior to loading, (2) the total resin binding capacity is 20 mg/mL, (3) the eluant volume is equal to 6 CVs, (4) the total volume of the solutions for column wash, regeneration, and storage is 15 CVs, (5) the protein of interest is recovered in 1 CV of eluant buffer with a recovery yield of 90%, and (6) the material of a batch is handled in three cycles.

Enzymatic Conversion. The removal of the C-peptide from human proinsulin is carried out enzymatically (using trypsin and carboxypeptidase B) in a reaction vessel (V-105). Trypsin cleaves at the carboxy-terminus of internal lysine and arginine residues, and carboxypeptidase B

removes terminal amino acids. The amount of trypsin used is rate limiting and allows intact human insulin to be formed. Carboxypeptidase is added to a final concentration of 4 mg/L, while trypsin is added to a final concentration of 1 mg/L. The reaction takes place at 30°C for 4 h and reaches a conversion yield of 95%. The volume of the solution at this point is around 4300 L.

Final Purification Section A purification sequence based on multimodal chromatography, which exploits differences in molecular charge, size, and hydrophobicity, is used to isolate biosynthetic human insulin. A description of all the purification steps follows.

The enzymatic conversion solution is exchanged with WFI and concentrated by a factor of 4 in a diafilter (DF-102). An ion exchange column (C-103) is used to purify the insulin solution. The following operating assumptions were made: (1) the column is equilibrated for 30 min prior to loading, (2) the total resin binding capacity is 20 mg/mL, (3) the eluant volume is equal to 8 CVs and the eluant is an 11.5% w/w solution of NaCl in WFI, (4) the total volume of the solutions for column wash, regeneration, and storage is 14 CVs, (5) the protein of interest is recovered in 1.5 CV of eluant buffer with a recovery yield of 95%, and (6) the material from each batch is handled in four cycles. The solution volume at this point is around 1780 L.

Next, the ion exchange eluant solution is exchanged with WFI in a diafilter (DF-103) and is concentrated by a factor of 2.0. A recovery yield of 98% was assumed for this step (2% denatures).

The purification of the insulin solution proceeds with a reversed-phase high performance liquid chromatography (RP-HPLC) step (C-104). Detailed information on the use of RP-HPLC for insulin purification is available in the literature. Analytical studies with a variety of reversed-phase systems have shown that an acidic mobile phase can provide excellent resolution of insulin from structurally similar insulin-like components. Minor modifications in the insulin molecule, resulting in monodesamido formation at the 21st amino acid of the A chain, or derivatization of amines via carbamoylation or formylation, result in insulin derivatives having significantly increased retention. Derivatives of this nature are typical of the kind of insulin-like components that are found in the charge stream going into the reversed-phase purification. The use of an acidic mobile phase results in the elution of all the derivatives after the insulin peak, while the use of mildly alkaline pH results in derivatives eluted on either side of the parent insulin peak. An ideal pH for insulin purification is in the region of 3.0 to 4.0, since this pH range is far enough below the isoelectric pH of 5.4 to provide for good insulin solubility. An eluant buffer

with an acetic acid concentration of 0.25 M meets these operational criteria because it is compatible with the chromatography and provides good insulin solubility. A 90% insulin yield was assumed in the RP-HPLC step with the following operating conditions: (1) the column is equilibrated for 30 min prior to loading, (2) the total resin binding capacity is 15 mg/ml, (3) the column height is 25 cm, (4) the eluant volume is 6 CVs and its composition is 25% w/w acetonitrile, 1.5% w/w acetic acid, and 73.5% w/w WFI, (5) the total volume of the solutions for column wash, equilibration, regeneration, and storage is 6 CVs, and (5) the protein of interest is recovered in 1 CV of eluant buffer with a recovery yield of 90%.

The RP-HPLC buffer is exchanged with WFI and concentrated by a factor of 2.0 in a diafilter (DF-104) that has a product recovery yield of 98% (2% denatures). Purification is completed by a gel filtration chromatography column (C-105). The following operating assumptions were made: (1) the column is equilibrated for 30 min prior to loading, (2) the sample volume is equal to 5% of the column volume, (3) the eluant volume is equal to 4 CVs, (4) the total volume of the solutions for column wash, depyrogenation, stripping, and storage is 6 CVs, and (5) the protein of interest is recovered in 0.5 CV of eluant buffer with a recovery yield of 90%. The mobile phase is a solution of acetic acid.

Next, a diafilter (DF-105) is used to concentrate the purified insulin solution by a factor of 10. The solution volume at this point is around 180 L, which contains approximately 12.8 kg of insulin. This material is pumped into a jacketed and agitated tank (V-106). Ammonium acetate and zinc chloride are added to the protein solution until each reaches a final concentration of 0.02 M [32]. The pH is then adjusted to between 5.4 and 6.2. The crystallization is carried out at 5°C for 12 h. Insulin crystallizes with zinc with the following stoichiometry: $insulin_6$-Zn_2. Step recovery on insulin is around 90%.

The crystals are recovered with a basket centrifuge (BCF-101) with a yield of 95%. Finally, the crystals are freeze-dried (FDR-101). The purity of the crystallized end product is between 99.5 and 99.9% as measured by analytical high performance liquid chromatography (HPLC). Approximately 11.5 kg of product is recovered per batch. The overall recovery yield is around 32%.

Material Balances and Environmental Impact Assessment

Table 11.12 displays the material requirements in kilograms per year, per batch, and per kilogram of main product (MP = purified insulin crystals). The solutions of H_3PO_4 (5% w/w) and

NaOH (0.5 M) are used for equipment cleaning. WFI is used for preparing all the buffers utilized in product purification as well as all the cleaning solutions. Note the large amounts of formic acid, urea, guanidine hydrochloride, acetic acid, and acetonitrile required per kilogram of final product. All these materials end up in waste streams.

TABLE 11.12

Raw Material Requirements for Human Insulin Production: 1 Batch = 11.5 kg Main Product (MP = Purified Insulin Crystals)

Raw material	Requirement		
	kg/year	kg/batch	kg/kg MP
Glucose	782,238	4,888	433.7
Salts	71,428	446	39.6
Water	9,715,481	60,721	5,386.9
H3PO4 (5% w/w)	3,979,049	24,869	2,206.3
NaOH (0.5 M)	3,842,379	24,014	2,130.5
WFI	51,885,267	324,282	28,768.8
Ammonia	81,553	509	45.2
EDTA	10,418	65	5.8
TRIS Base	43,162	269	23.9
Triton-X-100	3,035	18	1.7
MrEtOH	98,660	616	54.7
Urea	3,054,070	19,087	1,693.4
CNBr	15,268	95	8.5
Formic Acid	1,751,525	10,947	971.2
Guanidine-HCl	805,593	5,034	446.7
Na2O6S4	24,159	150	13.4
NH4HCO3	5,551	34	3.1
Sodium Sulfite	48,318	301	26.8
Sodium Chloride	775,549	4,847	430.0
Acetic-Acid	975,741	6,098	541.0
Sodium Hydroxide	137,239	857	76.1
Enzymes	3	0	0.0
Acetonitrile	764,739	4,779	424.0
Ammonium Acetate	181	1	0.1
Zinc Chloride	320	2	0.2
TOTAL	78,870,927	492,943	43,731.5

In the base case, this waste is treated and disposed. However, opportunities may exist for recycling some chemicals for in-process use and recovering others for off-site use. For instance, formic acid (HCOOH), acetonitrile, and urea are good candidates for recycling and recovery. Formic acid is used in large quantities (11 tons/batch) in the CNBr cleavage step (V-102), and it

is removed by means of a rotary vacuum evaporator (CSP-101), along with small quantities of CNBr, H_2O, and urea. The recovered formic acid can be readily purified by distillation and recycled in the process. Around 2.2 metric tons per batch of urea is used for the dissolution of inclusion bodies (V-101), and 17 metric tons per batch is used in the first chromatography step (C-101) to purify proinsulin$(S—SO3)_6$ before its refolding. Approximately 90% of the urea appears in just two waste streams (Liq Waste 7 and 8). It is unlikely that these urea-containing streams can be purified economically for in-process recycling. However, these solutions can be concentrated, neutralized, and shipped off site for further processing and utilization as a nitrogen fertilizer.

Approximately 4.8 metric tons (MT) per batch of acetonitrile is used in the reversed-phase HPLC column (C-104), and most of it ends up in the waste stream of the column (Liq Waste 13) along with 6.8 MT of water, 1.85 MT of acetic acid, and small amounts of NaCl and other impurities. It is unlikely that acetonitrile can be recovered economically to meet the high purity specifications for a step so close to the end of the purification train. However, there may be a market for off-site use.

Process Scheduling

Figure 11.15 displays the scheduling and equipment utilization for six consecutive batches. The batch time is approximately 11 days. This is the time required to go from the preparation of raw materials to final product for a single batch (excluding inoculum preparation). However, since most of the equipment items are utilized for much shorter periods within a batch, a new batch is initiated every 48 h. Multiple bars of the same color on the same line (e.g., for DS-101, DF-101, and DF-102) represent reuse (sharing) of equipment by multiple procedures. White space represents idle time. The equipment with the least idle time between consecutive batches is the *time* (*or scheduling*) *bottleneck* (V-104 in this case) that determines the maximum number of batches per year. Its occupancy time (approximately 45 h) is the minimum possible time between consecutive batches. The production line operates around the clock and processes 160 batches per year.

Process scheduling is closely related to the determination of the annual capacity of a batch process. The last part of this example discusses how changes in scheduling and installation of additional equipment can be used to increase process throughput and reduce manufacturing cost.

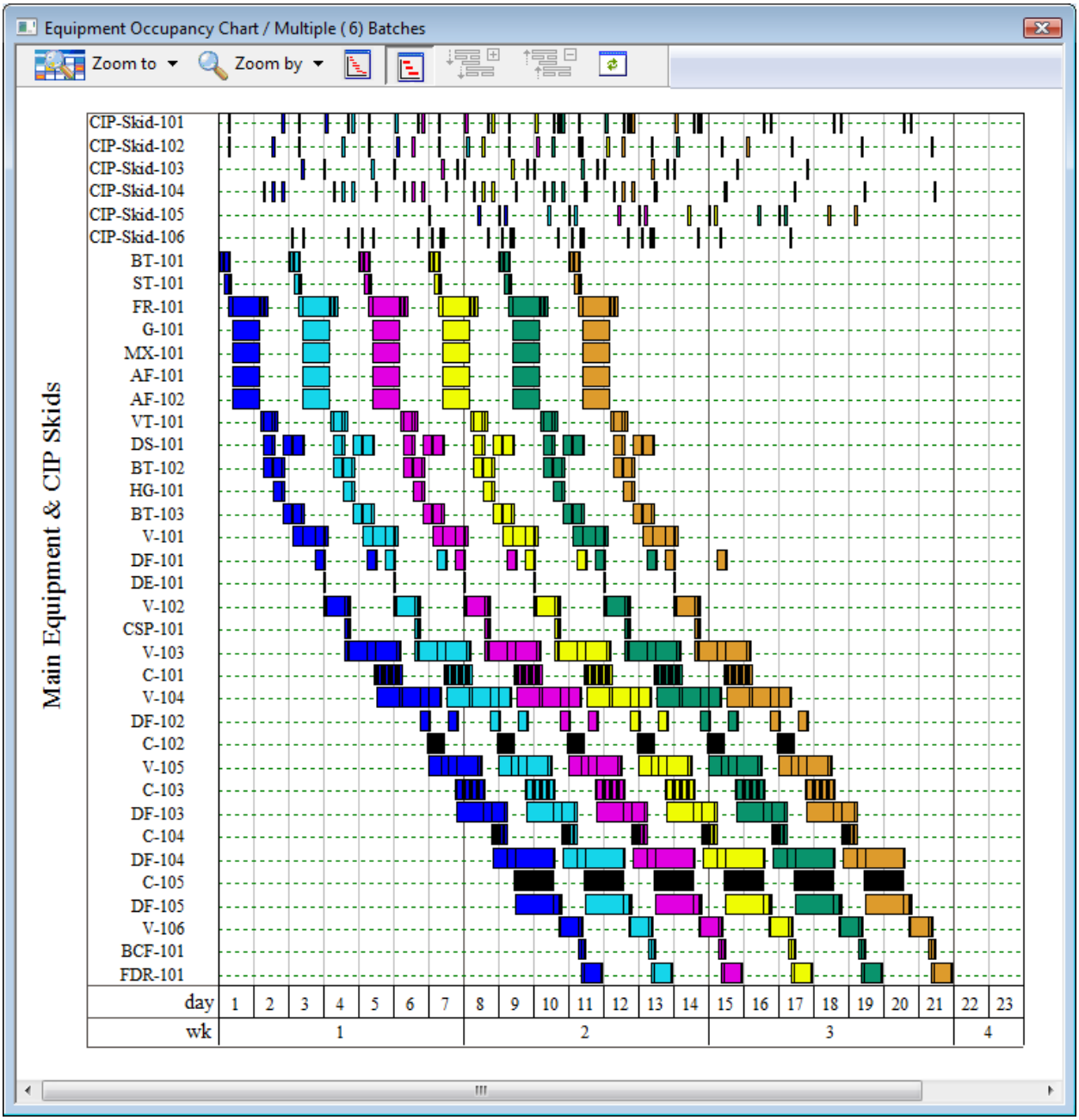

Figure 11.15 Equipment occupancy as a function of time for six consecutive batches.

Resource Tracking and Utility Sizing

Another characteristic of batch processing is the variable demand for resources (e.g., labor, utilities and raw materials) as a function of time. For instance, Figure 11.16 displays the demand for WFI for six consecutive batches. The red lines represent instantaneous demand, the blue lines represent averaged demand for 4-h intervals, and the green lines, which correspond to the y-axis on the right hand side of the chart, represent the cumulative demand for the same 4-h intervals.

These charts are useful for sizing utility systems during the design of new facilities. For instance, a WFI system consists of a still that generates distilled water, a surge tank, and a circulation loop for delivering the material around the plant. The chart of Figure 11.16 provides reasonable estimates for the size of the still, the surge tank, and the pumping capacity of the circulation loop. More specifically, the instantaneous demand peak (red line) indicates the minimum pumping capacity for the system since this capacity needs to meet the peak demand (47,500 kg/h). The cumulative demand peak (green line) can be used to size the surge tank, which must be large enough to maintain capacity during peak cumulative demand (in this case, the highest demand during any 4-hour interval is 55,000 kg). Finally, the highest averaged demand (blue line) indicates the size of the still (production capacity of 14,000 kg/h). The trade-off between still rate and surge capacity can be examined by changing the averaging time interval. For example, selecting an interval greater than 4 hours results in a larger surge tank and a lower still rate (e.g., a smaller still) compared to the base case.

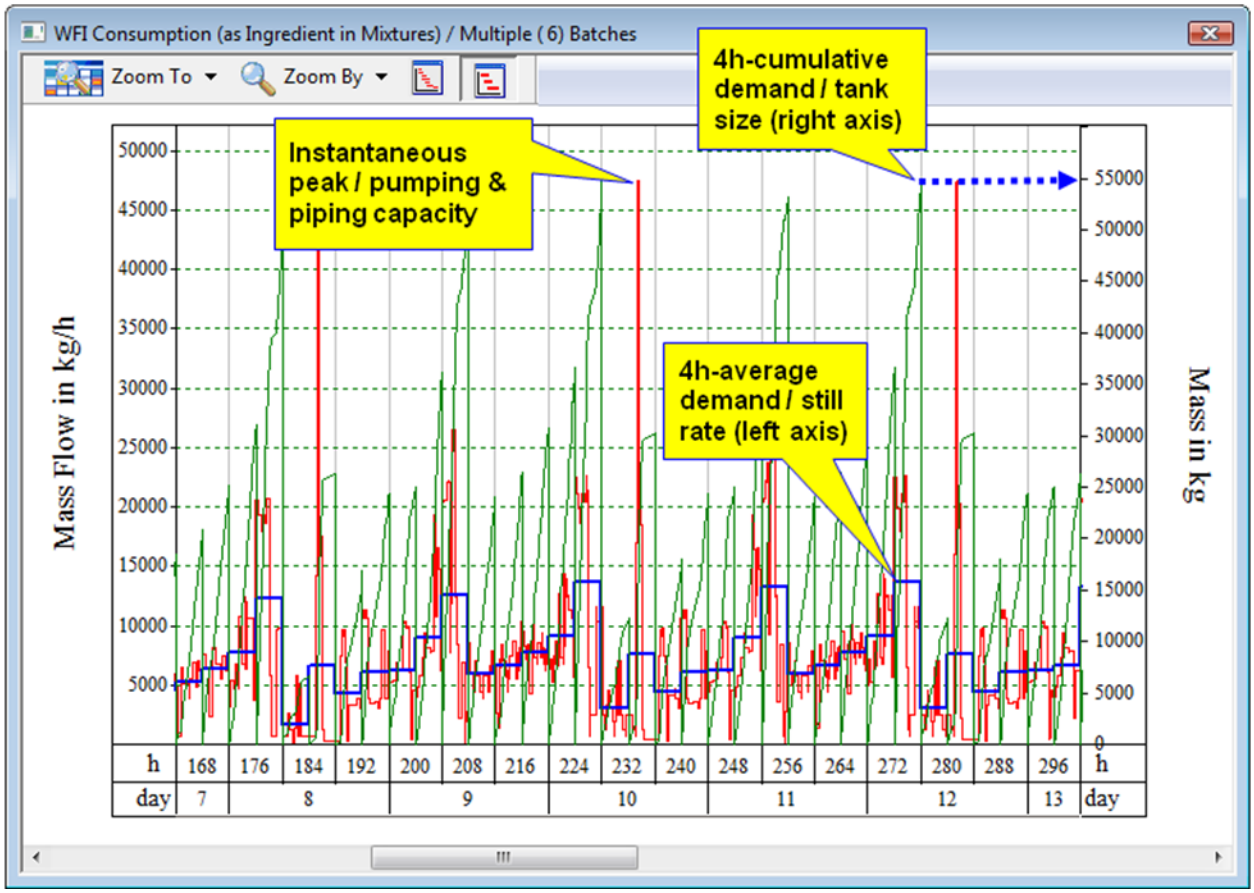

Figure 11.16 WFI demand as a function of time

Figure 11.17 displays the inventory profile of WFI in the surge tank (dark lines) for a tank size of 55,000 kg and a still rate of 14,000 kg/h (as indicated by the findings of Figure 11.16). The still is turned on when the WFI level falls below 35% of the tank's capacity. The still remains on until the tank is full. The operation rate and frequency of the still is depicted by the light blue step-function lines.

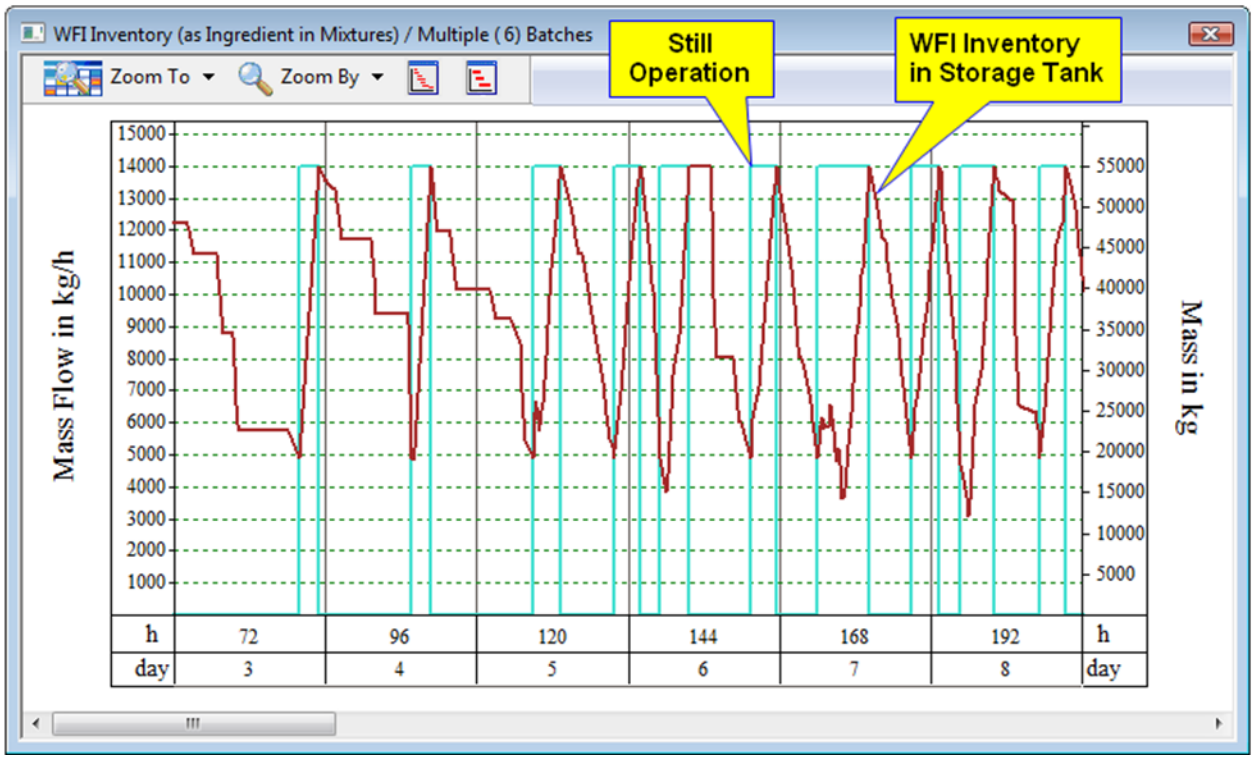

Figure 11.17 WFI inventory (dark lines) and operating frequency of still (light blue lines)

Sizing of bio-waste treatment systems can be handled in a similar manner. Such systems typically involve two tanks that alternate in operation periodically (while one is receiving, the other is treating a batch of waste material). The peak cumulative amount for the alternating period indicates the minimum capacity of each tank.

Economic Evaluation

Table 11.13 shows the results of the economic evaluation. The detailed tables for these calculations are available as part of the evaluation version of SuperPro Designer. For a plant of this capacity, the total capital investment is $178 million. The unit production cost is $61 per gram of purified insulin crystals. Assuming a selling price of $100/g, the project yields an after-

tax internal rate of return (IRR) of 35% and a net present value (NPV) of $250 million (assuming a discount interest rate of 7%). Based on these results, this project represents a very attractive investment. However, if amortization of up-front R&D costs is considered in the economic evaluation, the numbers change drastically. For instance, a modest amount of $100 million for up-front R&D cost amortized over a period of 10 years reduces the IRR to 16.8% and the NPV to $153 million.

TABLE 11.13

Key Economic Evaluation Results for Human Insulin Production

Direct fixed capital	$145 million
Total capital investment	$178 million
Plant throughput	1,803 kg/year
Manufacturing cost	$110 million/year
Unit production cost	$61/g
Selling price	$100/g
Revenues	$180 million/year
Gross profit	$70 million/year
Taxes (40%)	$28 million/year
IRR (after taxes)	35%
NPV (for 7% discount interest rate)	$250 million

Figure 11.18 breaks down the operating cost. The cost of consumables is the most important, accounting for 31.9% of the overall manufacturing cost. Consumables represent the expense of periodically replacing the resins of the chromatography columns and the membranes of the membrane filters. The cost of raw materials is the second most important, accounting for 27.3% of the overall cost. The facility-dependent cost is third, accounting for 24.1% of the overall cost. This cost item accounts for the depreciation and maintenance of the facility and other overhead expenses. Labor and Lab/QC/QA account for 9.1%. The treatment and disposal of waste materials accounts for 7.3% of the total cost. As mentioned in the material balance section, recycling and reuse of some of the waste materials may reduce this cost.

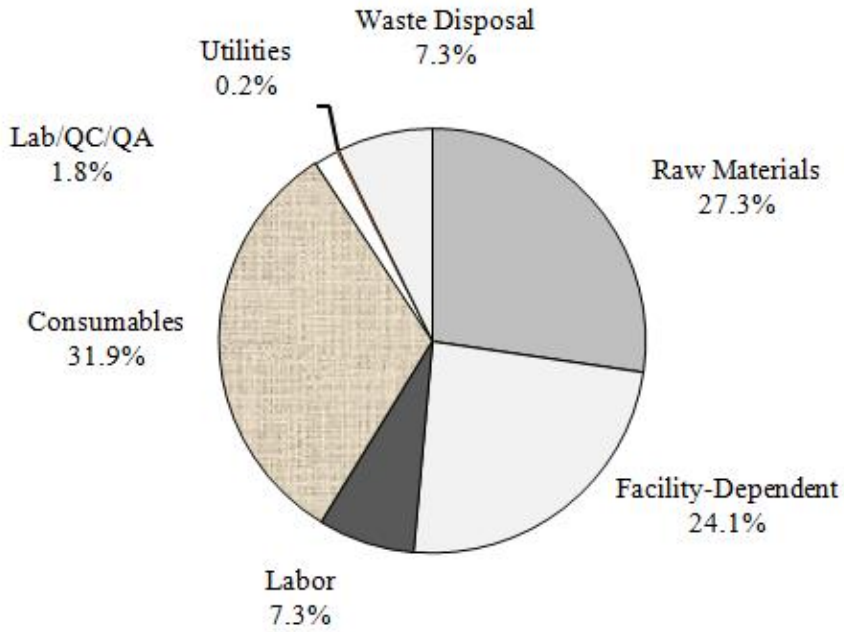

Figure 11.18 Breakdown of manufacturing cost for human insulin production

Figure 11.19 displays the percentage of the operating cost associated with each flowsheet section. Only 8.0% of the overall cost is associated with fermentation. The other 92% is associated with the recovery and purification sections. This is common for high value biopharmaceuticals that are produced from recombinant *E. coli*. Most of the cost is associated with the reactions section because of the large amounts of expensive chemicals and consumables required for purification.

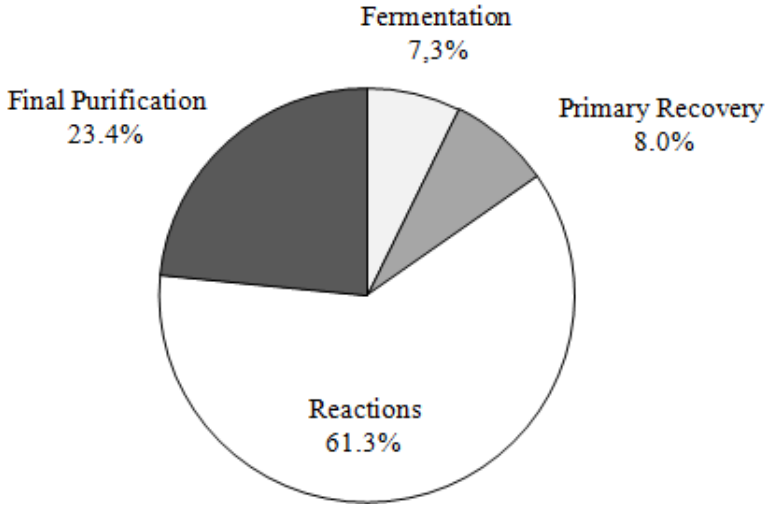

Figure 11.19 Cost distribution per flowsheet section for human insulin production

Finally, for each raw material used in the process, Table 11.14 displays the price, annual cost, and contribution to the overall raw materials cost. WFI, urea, and H_3PO_4 (5% w/w) are the top three contributors to the raw materials cost. The H_3PO_4 and NaOH solutions are used for equipment cleaning.

TABLE 11.14

Cost of Raw Materials for Human Insulin Production (Year 2012 Prices)

Bulk Material	Unit Cost ($/kg)	Annual Cost ($)	%
Glucose	0.60	469,343	1.56
Salts	1.00	71,428	0.24
Water	0.05	485,774	1.61
H3PO4 (5% w/w)	1.00	3,979,049	13.19
NaOH (0.5 M)	0.50	1,921,190	6.37
WFI	0.10	5,188,527	17.20
Ammonia	0.70	57,087	0.19
EDTA	18.50	192,734	0.64
TRIS Base	6.00	258,974	0.86
Triton-X-100	1.50	4,553	0.02
MrEtOH	3.00	295,980	0.98
Urea	1.52	4,642,186	15.38
CNBr	11.00	167,953	0.56
Formic Acid	1.60	2,802,441	9.29
Guanidine-HCl	2.15	1,732,025	5.74
Na2O6S4	0.60	14,495	0.05
NH4HCO3	1.00	5,551	0.02
Sodium Sulfite	0.40	19,327	0.06
Sodium Chloride	1.23	953,925	3.16
Acetic-Acid	2.50	2,439,353	8.08
Sodium Hydroxide	3.50	480,336	1.59
Enzymes	500,000.00	1,691,128	5.60
Acetonitrile	3.00	2,294,217	7.60
Ammonium Acetate	15.00	2,718	0.01
Zinc Chloride	12.00	3,840	0.01
TOTAL		30,174,133	100.00

Other assumptions for the economic evaluation include the following: (1) a new manufacturing facility will be built and dedicated to production of 1800 kg/year of purified insulin; (2) the entire direct fixed capital is depreciated linearly over a period of 10 years; (3) the project life-time is 15 years; (4) the unit cost of membranes is $\$800/m^2$ and they are replaced every 50 cycles; (5) the average unit cost of chromatography resins is \$1500/liter; (6) the waste disposal cost is $\$5/m^3$ for low BOD streams and $\$150/m^3$ for streams containing significant amounts of solvents and other regulated chemicals.

Throughput Increase Options

In the base case, a new batch is initiated every 48 h. Most of the equipment items, however, are utilized for less than 24 h per batch (see Figure 11.15). If the market conditions are favorable, this provides the opportunity for increasing plant throughput without major capital expenditures. A realistic improvement is to initiate a batch every 24 h. This will require a new fermentor of the same size as the original fermentor, whose operation will be staggered relative to the existing unit so that one fermentor is ready for harvesting every day. Such a production change will also require additional equipment of the following types: (1) disk-stack centrifuges to reduce the occupancy of DS-101 to less than 24 h, (2) two reaction vessels to reduce the occupancy of V-103 and V-104, and (3) membrane filters to reduce the occupancy of DF-104 and DF-105.

The additional capital investment for such a change is around $55 million. This additional investment will allow the plant's capacity to be doubled, and the new unit production cost will be around $55/g. The reduction in the unit production cost is rather small because the majority of the manufacturing cost is associated with consumables and raw materials that scale approximately linearly with production.

11.6.3 THERAPEUTIC MONOCLONAL ANTIBODY PRODUCTION

Monoclonal antibodies are large protein molecules that consist of two main regions, the Fab (Fragment Antigen Binding) region and the Fc (Fragment crystallizable) region. Monoclonal antibodies (MAbs) are the fastest-growing segment in the biopharmaceutical industry. MAbs are currently used to treat various types of cancer, rheumatoid arthritis, psoriasis, severe asthma, macular degeneration, multiple sclerosis, and other diseases. 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 [55 and 56]. The market size for MAbs in 2010 was in excess of $35 billion [57].

The high-dose demand for several MAbs translates into annual production requirements for purified product in the metric ton range. This example illustrates the analysis of a large scale MAb process. Again, the modeling and calculations are performed with SuperPro Designer.

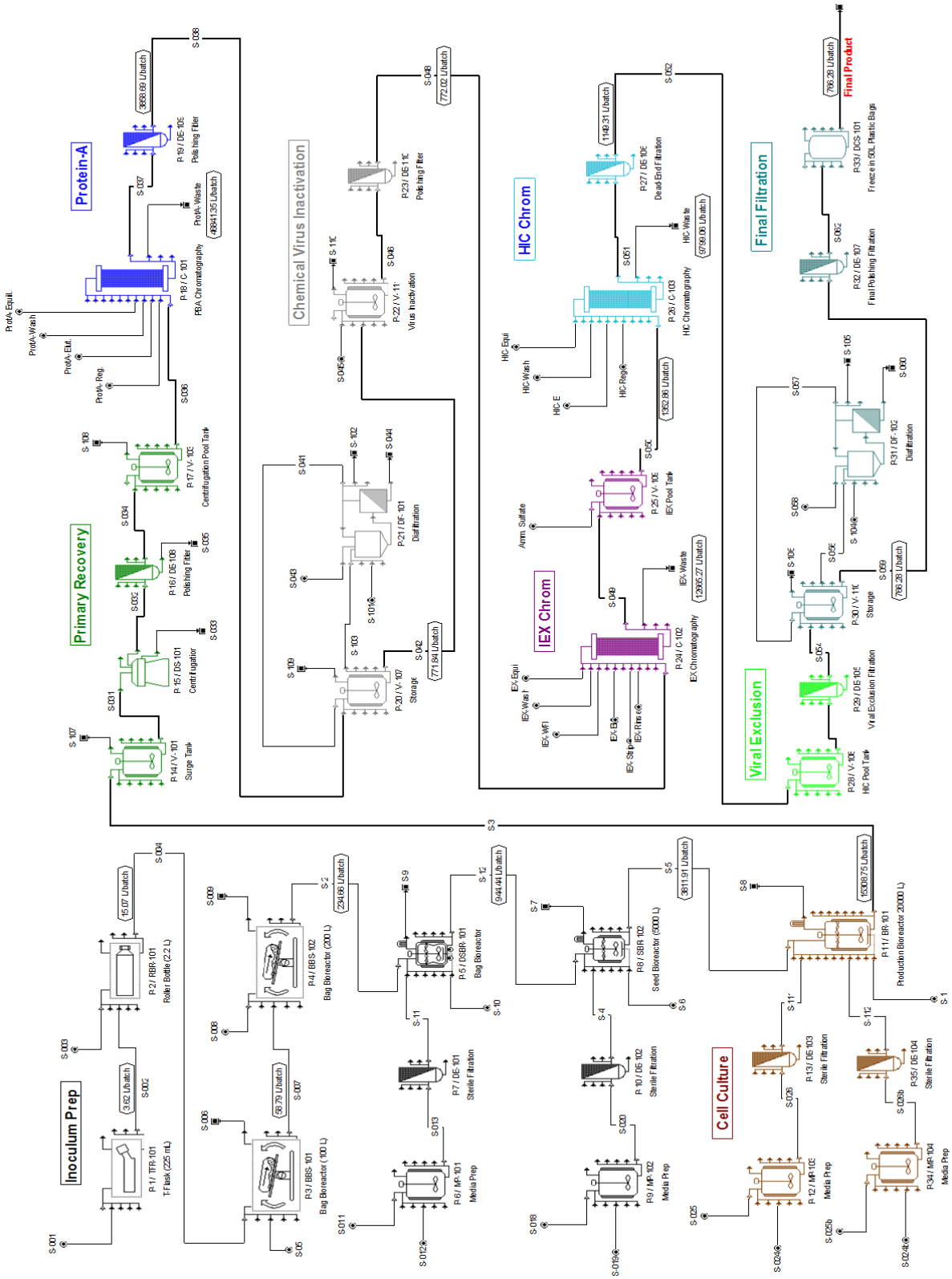

Figure 11.20 Monoclonal antibody production flowsheet.

Figure 11.20 displays the flowsheet of the overall process. The generation of the flowsheet was based on information available in the patent and technical literature combined with the authors' engineering judgment and experience with such processes [58]. The process in this example produces 1,544 kg of purified MAb per year. The flow diagram of Figure 11.20 is a simplified representation of the actual process because it lacks all the buffer preparation and holding activities. Such processes require 20-30 buffer solutions for product purification. These solutions are prepared in mixing tanks and then stored in holding tanks located close to the purification train. The tanks required for buffer preparation and holding add to the capital investment of the facility, while the required labor adds to the manufacturing cost. The model files for this example that are part of the evaluation version of SuperPro Designer (available at www.intelligen.com) include the tanks for buffer preparation and holding. In addition, the capital and operating costs associated with buffer preparation activities were considered in the cost analysis results presented in this example.

Process Description

Upstream Section The upstream part is split into two sections: the Inoculum Preparation section and the Bioreaction section. The inoculum is initially prepared in 225 mL T-flasks (TFR-101). Next, the material from the T-flasks is moved to 2.2 L roller bottles (RBR-101), then to 100 L and subsequently to 200 L rocking bioreactors that utilize disposable bags (BBS-101 and BBS-102). 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 stirred seed bioreactor (DSBR-101), which utilizes 1000 L disposable bags. The second seed bioreactor (SBR-102) is a 5000 L stainless steel vessel. For the two 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 & DE-102). In the cell culture 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 20,000 L stainless stirred-tank bioreactor (BR-101) is used to grow the cells, which produce the therapeutic monoclonal antibody (MAb). The production bioreactor operates in 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 dry media powder

in the initial feed solution is 17 g/L. The cell culture time is 12 days. The volume of broth generated per bioreactor batch is approximately 15,000 L, which contains roughly 30.5 kg of product (the product titer is approximately 2 g/L).

Downstream Section 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 5% of MAb is lost in the solids waste stream. The bulk of the contaminant proteins are removed using a Protein-A affinity chromatography column (C-101) which processes a batch of material in four cycles. The following operating assumptions were made for each chromatography cycle: (1) resin binding capacity is 15 g of product per L of resin, (2) 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), (3) the product is recovered in 2 CVs of eluant with a recovery yield of 90%, and (4) the total volume of the solution required for column equilibration, wash and regeneration is 14 CVs. The entire procedure takes approximately 27 h and requires a resin volume of 502 L. The protein solution is then concentrated 5-fold and diafiltered with two volumes of buffer (in P-21 / DF-101). This step takes approximately 8.4 h and requires a membrane of 21 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). The Ion Exchange (IEX) chromatography step (P-24 / C-102) that follows processes one batch of material in three cycles. The following operating assumptions were made for each cycle: (1) the resin's binding capacity is 40 g of product per L of resin, (2) 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, (3) the product is recovered in 2 CVs of eluant buffer with a MAb yield of 90% and (4) the total volume of the solutions required for column equilibration, wash, regeneration and rinse is 16 CVs. The step takes approximately 22.3 h and requires a resin volume of 210 L. Ammonium sulfate is then added to the IEX eluate (in P-25 / V-109) to a concentration of 0.75 M. This increases the ionic strength of the eluate in preparation for the Hydrophobic Interaction Chromatography (HIC, P-26 / C-103) step that follows. Like the IEX step which preceded it, the HIC step processes one batch of material in three cycles. The following operating assumptions were made for each cycle of the HIC step: (1) the resin binding capacity is 40 g of product per L of resin, (2) 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,(3) the product is

recovered in 2 CVs of eluant buffer with a recovery yield of 90% and (4) the total volume of the solutions required for column equilibration, wash and regeneration is 12 CVs. The step takes approximately 22 h and requires a resin volume of 190 L. A viral exclusion step (DE-105) follows. It is a dead-end type of filter with a pore size of 0.02 μm. 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 20 h and requires a membrane area of 10 m^2. The approximately 800 L of final protein solution is stored in twenty 50 L disposable storage bags (DCS-101). The overall yield of the downstream operations is 63.2%, and 19.3 kg of MAb are produced per batch.

Process Scheduling and Cycle Time Reduction

Figure 11.21 displays the equipment occupancy chart of the process for four consecutive batches. The schedule represents a plant that has a single production train. The CIP skids, polishing filters and media preparation tanks are not displayed on the chart for the sake of simplicity. 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 (BR-101) is the time (scheduling) bottleneck. On an annual basis, the plant processes 20 batches and produces approximately 386 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 batches—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 11.22 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 1,544 kg of MAb per year. Some biopharmaceutical companies have installed more than four bioreactor trains per purification train in order to achieve cycle times as low as two days.

Material Requirements

Table 11.15 provides a summary of the material requirements of the process. Note the large amount of WFI utilized per batch. The majority of WFI is consumed for cleaning and buffer preparation.

Economic Evaluation

Table 11.16 displays the key economic evaluation results generated using the built-in cost functions of SuperPro Designer. The total capital investment (for the case with the four bioreactor trains) is around $477 million. The total annual operating cost is $130 million, resulting in a unit production cost of around $84/g (1,544 kg of purified MAb are produced annually). Assuming a selling price of $200/g, the project yields an after-tax internal rate of return (IRR) of 24.3% and a net present value (NPV) of $560 million (assuming a discount interest rate of 7%).

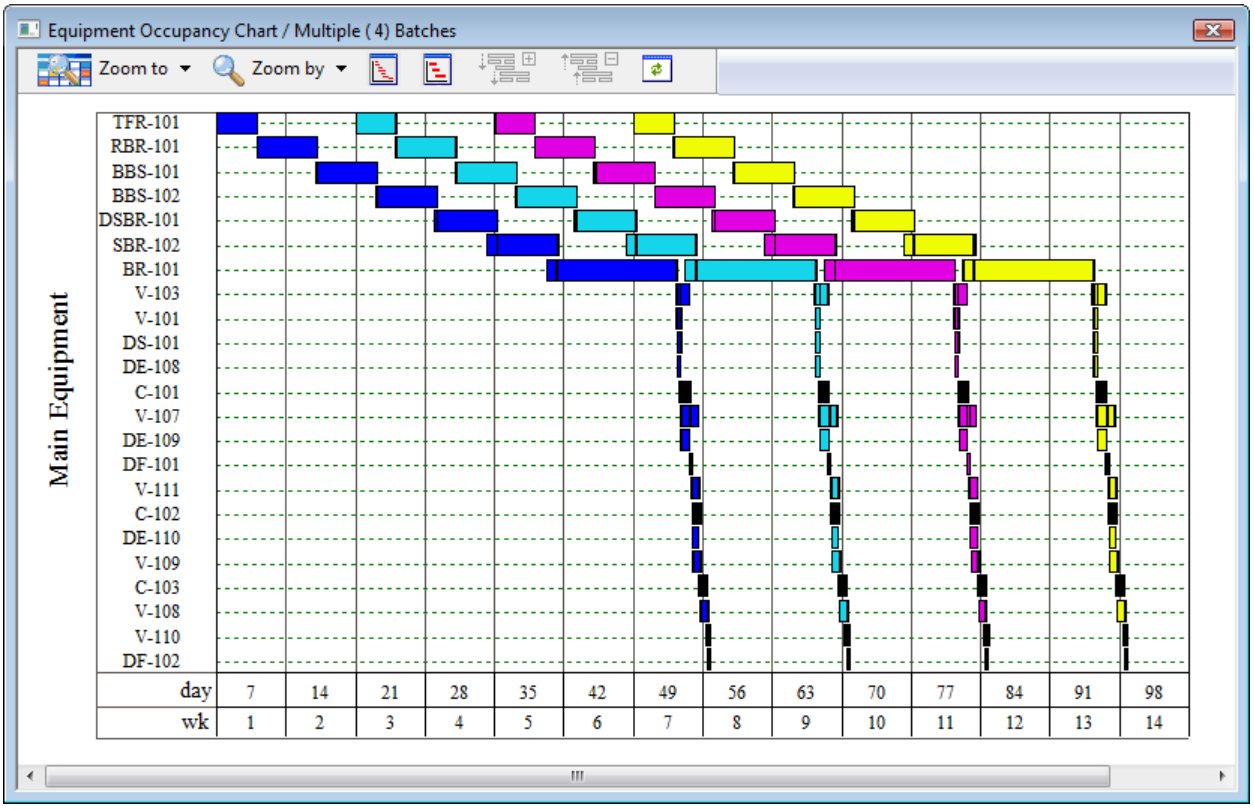

Figure 11.21 One bioreactor train feeding one purification train.

Table 11.17 presents a breakdown of the operating cost contributors. The facility-dependent cost is the most important item, accounting for 46.7% of the manufacturing cost or $39.2/g of final product. This is common for high value products that are produced in small quantities in expensive facilities. Depreciation of the fixed capital investment and maintenance of the facility are the main contributors to this cost. Consumables are the second most important operating cost, accounting for 18.2% of the total or $15.3/g of final product. Consumables include chromatography resins, membrane filters and disposable bags that need to be replaced on a

regular basis. Labor and raw materials costs come third and fourth, accounting for 14.6% and 12.9% of the total cost, respectively. The Miscellaneous cost item (4.2% of total) accounts for heating/cooling utilities, electricity, and environmental costs. The cost of WFI, commonly classified as a utility cost in industry, is accounted for in the cost of raw materials in this example. In terms of cost distribution per section, 62% of the cost is associated with the upstream section and 38% with the downstream.

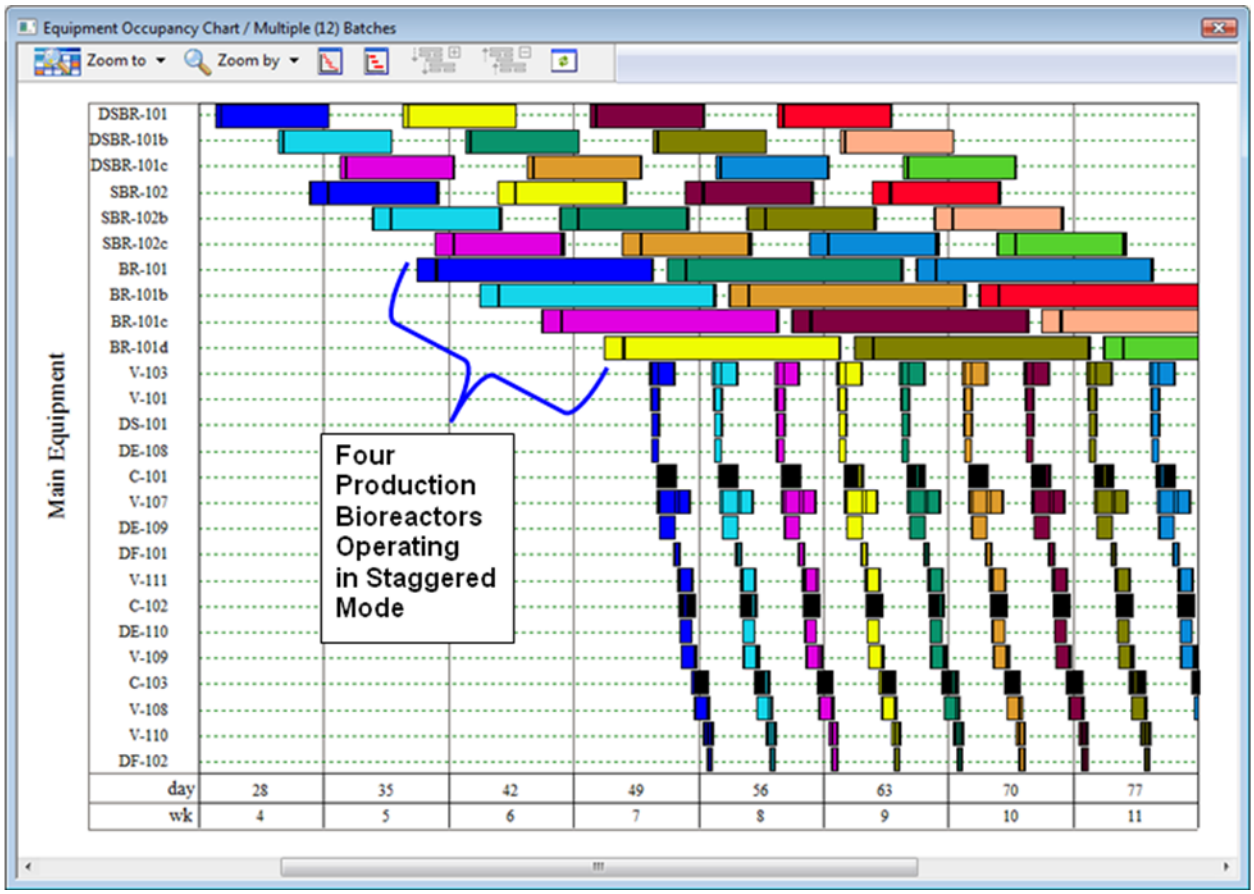

Figure 11.22 Four bioreactor trains feeding one purification train.

The economic evaluation relies on the following key assumptions: (1) a new manufacturing facility will be built and dedicated to production of 1,544 kg/year of MAb; (2) the entire direct fixed capital is depreciated linearly over a period of 12 years; (3) the project lifetime is 16 years; (4) the unit cost of WFI is \$0.15/L; (5) the cost of the serum free media (in powder form) is \$300/kg; (6) all the chemicals used are high purity grade; (7) the unit cost of membranes is \$400/m^2; (8) the unit cost of chromatography resins is \$6000/L, \$1200/L, and \$2050/L for columns C-101, C-102, and C-103, respectively; (9) the chromatography resins are replaced

every 60, 50, and 50 cycles for columns C-101, C-102, and C-103, respectively.

TABLE 11.15

Raw Material Requirements (MP= Purified MAb)

Material	kg/yr	kg/batch	kg/kg MP
Inoc Media Sltn	18,626	232.82	12.060
WFI	8,429,839	105,372.99	5,458.171
SerumFree Media	37,343	466.79	24.179
H_3PO4 (5% w/w)	2,277,348	28,466.85	1,474.542
NaOH (0.5 M)	2,054,476	25,680.96	1,330.237
NaOH (0.1 M)	7,814,884	97,686.06	5,059.999
Amm. Sulfate	12,163	152.04	7.875
Polysorbate 80	6	0.08	0.004
Protein A Equil-Buffer	1,967,126	24,589.08	1,273.679
Protein A Elution-	800,194	10,002.42	518.111
Prot-A Reg Buffer	480,387	6,004.84	311.042
NaCI (1 M)	184,181	2,302.27	119.254
IEX-El-Buffer	16,133	201.67	10.446
IEX-Eq-Buffer	664,906	8,311.33	430.515
HIC-El-Buffer	239,200	2,990.00	154.877
HIC-Eq-Buffer	449,633	5,620.41	291.129
Concentrated PBS	14,371	179.64	9.305
EtOH (10% w/w)	362,998	4,537.48	235.035
TOTAL	**25,823,814**	**322,797.71**	**16,720.46**

TABLE 11.16

Key Economic Evaluation Results for MAb Production

Direct fixed capital	$365 million
Total capital investment	$477 million
Plant throughput	1,544 kg of MAb/year
Manufacturing cost	$130 million/year
Unit production cost	$84/g of MAb
Selling price	$200/g of MAb
Revenues	$309 million/year
Gross profit	$179 million/year
IRR (after taxes)	24.3%
NPV (for 7% discount interest rate)	$560 million

TABLE 11.17

Breakdown of the manufacturing cost for MAb production

Cost Item	$million/yr	$/g	%
Raw Materials	16.67	10.8	12.86
Facility-Dependent	60.54	39.2	46.71
Labor	18.89	12.2	14.58
Consumables	23.58	15.3	18.19
Lab/QC/QA	4.45	2.9	3.43
Miscellaneous	5.47	3.5	4.23
TOTAL	**129.60**	**83.9**	**100.00**

Sensitivity Analysis

After a model of the entire process has been developed on the computer, tools like SuperPro Designer can be used to ask and readily answer "what if" questions and to carry out sensitivity analysis with respect to key design variables. In this example, we looked at the impact of product titer (varied from 1 to 10 g/L) and bioreactor size (10,000 and 20,000 L) on unit production cost. Figure 11.23 displays the results of the analysis. All points correspond to four production bioreactors feeding a single purification train. For low product titers, the bioreactor volume has a considerable effect on the unit production cost. For instance, for a product titer of 1 g/L, going from 10,000 L to 20,000 L of production bioreactor volume reduces the unit cost from $230/g to $162/g. On the other hand, for high product titers (e.g., around 5 g/L), the impact of bioreactor scale is not as important. This can be explained by the fact that at high product titers, the majority of the manufacturing cost is associated with the purification train. It is therefore wise to shift R&D efforts from cell culture to product purification as the product titer in the bioreactor increases. A key assumption underlying the sensitivity analysis is that the composition and cost of the cell culture media is independent of product titer.

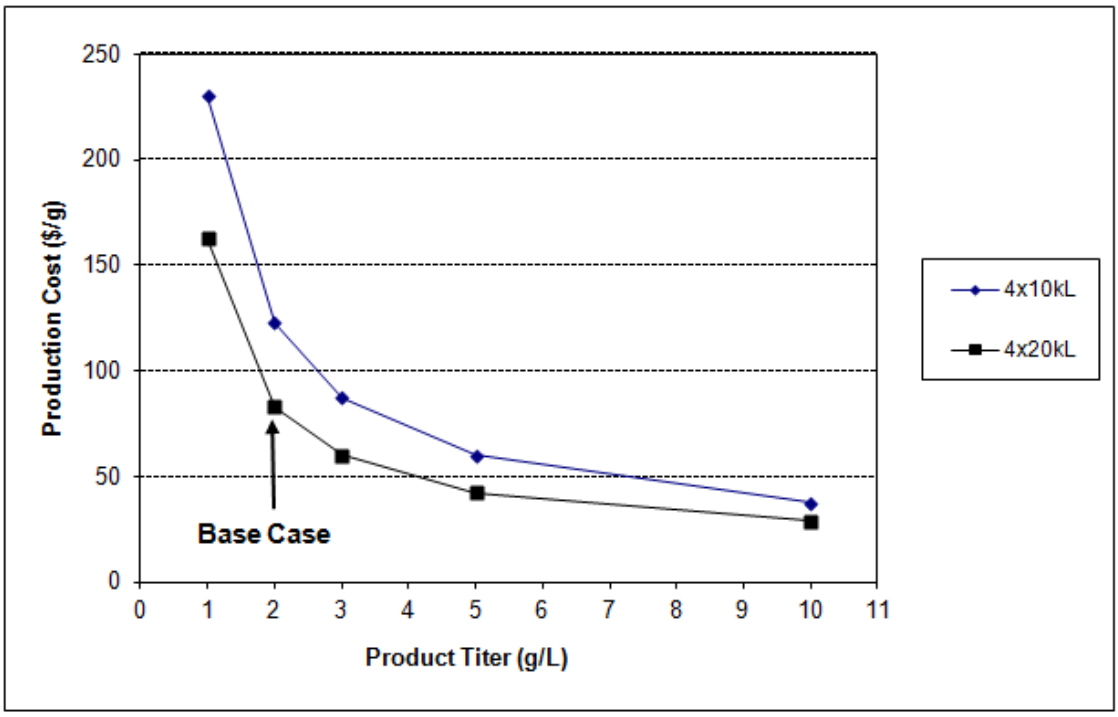

Figure 11.23 MAb production cost as a function of product titer and production bioreactor volume

11.7 Summary

Process design consists of two main activities, process synthesis and process analysis. Process synthesis is the selection and arrangement of a set of unit operations (process steps) capable of producing the desired product at an acceptable cost and quality. Process analysis is the evaluation and comparison of different process synthesis solutions. In general, a synthesis step is usually followed by an analysis step, and the results of the analysis determine the subsequent synthesis step. Process synthesis and analysis require integration of knowledge from many different scientific and engineering disciplines, and they are carried out at various levels of detail. Some important points to remember when performing process synthesis and analysis are:

- Sequencing of product purification steps is based on experience and rules of thumb (heuristics), in which biomass separation (harvesting or removal) is followed by extraction (isolation) of product and then purification and polishing.
- Process simulators facilitate the analysis of integrated processes by performing material and energy balances, equipment sizing, economic evaluation, cycle time analysis, environmental impact assessment and other tasks.

- Capital costs are estimated based on direct fixed capital, working capital, and start-up and validation costs.
- The cost of a processing step is the sum of the costs of raw materials, labor, consumables, quality control, waste treatment/disposal, utilities, equipment depreciation, equipment maintenance, and overhead. The cost of the entire process is the sum of the costs for all the steps.
- Profitability analysis consists of the calculation of gross margin, return on investment, payback time, net present value, internal rate of return and other measures.
- Sensitivity analysis uses a process simulator to investigate the impact of changing a specific process variable, such as product titer or scale of production. This activity can help to focus process design or development resources on areas of the process that are likely to have the greatest economic impact.

PROBLEMS

11.1 **Tissue Plasminogen Activator** Tissue plasminogen activator (tPA) was among the first products of biotechnology to be based on recombinant DNA technology. A medium molecular weight enzymatic protein, tPA is primarily used in the treatment of myocardial infarction (heart attack) patients. First characterized in 1979, the protein was commercially developed by Genentech (now part of Roche Pharmaceuticals), with clinical trial quantities and purities being achieved in late 1984. The current market demand is around 25 kg/year of purified tPA, which generates annual revenues of around $500 million for Roche Pharmaceuticals. The typical dose of tPA is around 100 mg, and this corresponds to a price per dose of around $2000.

The management of your company believes that the annual world demand can increase to more than 200 kg if the price per dose is reduced to around $300. Before committing to the venture, your president would like you, as director of corporate planning, to evaluate a technology for using transgenic goats to produce 50 kg of tPA per year. Based on data from GTC Biotherapeutics, you know that you can buy milk containing tPA at a concentration of 20 g/L for around $200/g of tPA.

Based on information from the technical and patent literature, develop and evaluate a process that can recover and purify 50 kg of tPA per year from goat milk.

More specifically, estimate the capital investment required and the profitability of the project idea.

11.2 **Indigo** Indigo is a dye that is used by denim manufacturers (to make blue jeans). It has traditionally been produced through chemical synthesis. The chemical route, however, generates large amounts of regulated waste materials that make the process environmentally unattractive. In the late 1990s, Genencor International (a biotechnology company which is now part of Du Pont) commercialized a technology for producing indigo via fermentation.

Based on information from the technical and patent literature, develop and evaluate a process for producing 5,000,000 kg of indigo per year via fermentation. The product must meet the quality specifications of the denim industry. More specifically, estimate the capital investment required and the unit production cost.

11.3 **L-Lysine** L-Lysine is an amino acid that is produced in large quantities (>100,000 metric tons/year) via fermentation. It is used as an animal feed supplement mainly for poultry and pigs.

Based on information from the technical and patent literature, develop and evaluate a process for producing 15,000,000 kg of L-lysine per year via fermentation. Your analysis should include estimation of capital and operating costs.

11.4 **Xanthan Gum** Xanthan gum is a water-soluble polysaccharide produced via fermentation. It is used in food products as a thickener, stabilizer, and emulsifier. Xanthan gum is also used for enhanced oil recovery.

Based on information from the technical and patent literature, develop and evaluate a process for producing 10,000,000 kg of xanthan gum per year. The product should meet the specifications of the petroleum industry for enhanced oil recovery. Your analysis should include estimation of capital and operating costs. Also, perform a sensitivity analysis and estimate the unit production cost for plant capacities ranging from 10 to 50 million kg of xanthan gum per year.

11.5 **Biodegradable Polymers** Because of the capacity limitations of urban landfills, biodegradable plastic packaging materials are of interest as a means to reduce the load on solid waste disposal systems.

Poly-2-hydroxybutyrate (PHB) is a promising biodegradable polyester that can be

produced via fermentation. Microorganisms that synthesize PHB include Gram-positive and Gram-negative species and cyanobacteria. Some members of the *Alcaligenes* and *Azotobacter* genera are the most promising because they store high levels of PHB. PHB is synthesized and stored intracellularly as a possible future carbon and energy source. High levels of polymer are obtained under nitrogen and phosphorus limitation. At optimum conditions, PHB can reach 70 to 80% by weight of the cell mass of the organism.

Based on information from the technical and patent literature, design and evaluate a plant that produces 30,000,000 kg of PHB per year. Your analysis should include estimation of capital and operating costs.

11.6 Laundry Enzymes Proteolytic enzymes are used in laundering to hydrolyze and remove proteinaceous stains. The commercially important proteolytic enzymes that are used in detergents are mainly produced by *Bacillus subtilis* and *Bacillus licheniformis*. These enzymes are endo-cleaving, have broad specificity, and are active over a wide pH range; calcium improves their stability at high temperature or extremes of pH. Their molecular weight is around 30,000 and their isoelectric point is in the range of 8.5 to 9.5.

The current world demand for laundry enzymes is around 10,000 metric tons (of pure enzyme) per year, corresponding to a world market of around $300 million.

The marketing department of your company believes that the annual world demand can increase to more than 20,000 metric tons if the selling price is reduced to around $15/kg of pure enzyme. Before committing to the venture, your company would like you to evaluate the cost structure of the current producers and find out whether it is possible (through the use of genetic engineering and modern separation technologies) to produce such enzymes for less than $10/kg.

11.7 Therapeutic Proteins from Transgenic Tobacco Transgenic plants (e.g., corn, tobacco, etc.) have the potential to produce complex bioactive proteins at significantly lower cost than production via transgenic animals or mammalian cell cultures. The advantages of transgenic plant production are: easy and efficient introduction of stable foreign genes, cost-effective biomass production ($0.04–$0.1/kg), no possible contamination with human disease agents, and the ability to perform the complex protein processing needed for many bioactive human therapeutics. Cost-effective biomass production makes this

mode of production suitable for high-volume recombinant proteins. The ability to perform complex protein processing is advantageous for production of therapeutic glycoproteins and bioactive peptides. Downstream processing costs are a major portion of the total unit production cost associated with transgenic plant production of high-volume therapeutic proteins. Therefore, primary recovery requires significant volume reduction. Once volume reduction and biomass removal have been achieved, chromatographic purification is required to remove plant protein impurities.

Design a purification process for use in a facility manufacturing 100 metric tons per year of recombinant human serum albumin protein from transgenic tobacco. Assume that the expression level is 0.5 g of product protein per kilogram of tobacco. Assume that biomass production and primary recovery are performed at a separate site. The feed to the purification section is 4000 L/day (containing 200 g/L of product protein), and this material is purchased for $0.7/g of product protein. The process should include the appropriate filtration and chromatographic steps. Assume that a combination of affinity and ion exchange chromatography will provide a product that is more than 90% pure.

11.8 **Succinic Acid** In a report published by the U.S. Department of Energy [59], succinic acid was identified as one of the top building-block chemicals that could be produced from renewable biomass. Currently, succinic acid uses a petroleum-derived maleic anhydride route for its production, which is costly and environmentally unfriendly. As a result, there is growing interest in new production technologies that are based on fermentation. There are several strains of bacteria which can efficiently transform glucose and other sugars into succinic acid, but separation of the product from the fermentation broth is difficult. Various methods of succinic acid recovery have been proposed, including ion exchange chromatography, precipitation with calcium hydroxide, liquid-liquid extraction with tri-*n*-octylamine, electrodialysis, etc.

Based on information from the technical and patent literature, design and evaluate a process for producing 30,000,000 kg of succinic acid per year via fermentation. Your analysis should include overall material and energy balances, equipment sizing, and estimation of capital and operating costs.

11.9 **Riboflavin (Vitamin B_2)** Riboflavin is used for human nutrition and therapy, and as an animal feed additive. Its use in animal nutrition helps to keep the animals healthy and fit.

Chemical synthesis of riboflavin was replaced by fermentation processes in the 1990s. The annual world demand for riboflavin was around 5,000,000 kg in 2010. Approximately 75% is consumed as an animal feed additive and the remainder is used for human food and pharmaceutical applications.

Based on information from the technical and patent literature, design and evaluate a fermentation process for producing 1,000,000 kg of riboflavin per year for food and pharmaceutical applications. Your analysis should include overall material and energy balances, equipment sizing, and estimation of capital and operating costs.

11.10 Cheese Whey In whey production, proteins are isolated from the liquid part of the milk that is separated from the curd when making cheese. Whey protein is often sold as a nutritional supplement for protein drinks, energy bars, etc. Such supplements are especially popular in the sport of bodybuilding. Whey proteins also play a vital role in the formulation of amino acid and protein profile of infant formulas, making them nutritionally similar to mother's milk and enhancing their nutritional value to infants. Whey protein is a mixture of the following individual protein components: Beta Lactoglobulin, Alpha-lactalbumin, Immunoglobulins, Glycomacropeptide, Lactoferrin, Lactoperoxidase, Lysozyme, Bovine Serum Albumin. The U.S. market for whey proteins is expected to exceed 200,000,000 kg by 2015.

Fresh whey from conventional cheese production is approximately 94% water, less than 1% protein, 4.5% lactose, less than 1% ash and less than 1% fat. The separation of proteins from lactose and other low molecular weight components is accomplished using ultrafiltration systems. Isolation of individual protein components is accomplished with a combination of chromatography and membrane filtration units. Concentration of the lactose solution is done with reverse osmosis units.

Design a plant that processes 200,000 L/day of cheese whey and produces whey protein and purified lactose for infant formula.

11.11 Stevia Sweetener Stevia is a leafy green plant native to subtropical and tropical regions (http://en.wikipedia.org/wiki/Stevia). It is grown around the world for its sweet leaves, which are used in a variety of forms as a high-intensity sweetener. Stevia extracts have 200-300 times the sweetness of sugar and do not raise blood glucose. Stevia-based sweeteners are used today in dairy products, health drinks and carbonated beverages.

Both Coca Cola and PepsiCo have introduced drinks containing stevia-based sweeteners under the commercial names of Truvia and PureVia, respectively. Stevia sweeteners are presently extracted from the leaves of stevia plants. However, recent advances in synthetic biology have enabled the production of stevia sweeteners via fermentation.

Based on information from the technical and patent literature, design and evaluate a fermentation process for producing 5,000,000 kg of stevia sweeteners per year for applications in the beverage industries. Assume that production is accomplished via fermentation in yeast that secretes stevia molecules, reaching a product titer in the fermentation broth of 75 g/L. The purification train includes a disk-stack centrifuge for biomass removal and a sequence of chromatography, membrane filtration, crystallization, and drying units for the isolation and purification of the product molecule(s). Your analysis should include overall material and energy balances, equipment sizing, and estimation of capital and operating cost. Furthermore, estimate the profitability of the investment assuming a selling price for the final product equal to 200 times the current price of sucrose.

11.12 MAb Production in Stirred Tank Bioreactors with Disposable Bags The MAb example in section 11.6.3 analyzes a process for producing 1,544 kg of purified MAb per year using four 20,000 L stainless steel production bioreactors operating in staggered mode (out of phase) and feeding a single purification train. The product titer is 2 g/L and the cycle time of each bioreactor is 2 weeks. In the last few years, new cell lines have become available (e.g., PER.C6 from Percivia, LLC) that can reach significantly higher product titers (> 20 g/L). Deployment of such cell lines greatly reduces the volume of the upstream equipment and enables single-use systems to produce metric ton quantities of MAbs. Rocking and stirred tank bioreactors that utilize single-use (disposable) liners (bags) have become popular in the biopharmaceutical industry because they eliminate the need for cleaning and sterilization-in-place. Other advantages of such systems include increased processing flexibility and shorter validation, start-up, and commercialization times. Single-use bioreactors are available with working volume of up to 2,000 L.

Design a process using the new technologies described above which can produce 1,200 kg of a therapeutic monoclonal antibody per year. Assume you make use of the PER.C6 cell line that can consistently reach 10 g/L of product titer. For product

purification, assume that you need two adsorptive chromatography steps (e.g., affinity and hydrophobic interaction) followed by a polishing ion exchange membrane adsorber that operates in flow-through mode (the product flows through the unit but certain DNA and other charged impurity molecules are retained by the membrane). Your analysis should include overall material and energy balances, equipment sizing, and estimation of capital and operating costs.

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Figure Captions:

Figure 11.1 Types of design estimates during the life cycle of a product [2].

Figure 11.2 Generalized block diagram of downstream processing [5].

Figure 11.3 Benefits of using process simulators.

Figure 11.4 A flowsheet on the main window of SuperPro Designer.

Figure 11.5 Window for adding operations to a unit procedure using SuperPro Designer.

Figure 11.6 Dialog window of the elution operation.

Figure 11.7 Purchase cost of disk-stack centrifuges vs. Σ factor (2012 prices).

Figure 11.8 Purchase cost of membrane filtration systems (2012 prices).

Figure 11.7 Purchase cost of high pressure homogenizers versus throughput (1998 prices).

Figure 11.9 Purchase costs of chromatography columns made of acrylic tube and stainless steel bed supports (2012 prices).

Figure 11.10 Purchase cost of agitated tanks made of stainless steel (2012 prices).

Figure 11.11 Citric acid production flowsheet.

Figure 11.12 Equipment occupancy chart for 14 consecutive batches of the citric acid process.

Figure 11-13 Human insulin from proinsulin fusion protein.

Figure 11.14 Insulin production flowsheet.

Figure 11.15 Equipment occupancy as a function of time for six consecutive batches.

Figure 11.16 WFI demand as a function of time

Figure 11.17 WFI inventory (dark lines) and operating frequency of still (light blue lines)

Figure 11.18 Breakdown of manufacturing cost for human insulin production

Figure 11.19 Cost distribution per flowsheet section for human insulin production

Figure 11.20 Monoclonal antibody production flowsheet.

Figure 11.21 One bioreactor train feeding one purification train.

Figure 11.22 Four bioreactor trains feeding one purification train.

Figure 11.23 MAb production cost as a function of product titer and production bioreactor volume