TISSUE PLASMINOGIN ACTIVATOR PROCESS

Supplement to Chapters 1-2 of
Process Design Principles: Synthesis, Analysis, and Evaluation

This supplement is based upon the design carried out by Melissa Audette, Chris Metallo, and Kasidit Nootong in ChE 459 during the Spring 2000. Professor Scott Diamond prepared the design problem statement and served as the design group's advisor. Over the past summer, Matt Fucci assisted in the preparation of these materials. Note that the supplement to Chapter 3 on *Principles of Batch Process Simulation* is provided separately.

June 18, 2002
W. D. Seider

1.2 STEPS IN DESIGNING AND RETROFITTING CHEMICAL PROCESSES.

Assess Primitive Problem (add following last paragraph on page 8)

Another primitive problem, in pharmaceuticals manufacture, involves plasminogen activators, which are powerful enzymes that trigger the proteolytic degradation of blood that cause strokes and heart attacks. Since the mid-1980s, Genentech has manufactured tissue plasminogen activator (tPA), which they currently sell for $2,000 per 100 mg dose, with annual sales of $300 MM/yr. Given that their patent will expire soon, Genentech has developed a next generation, FDA-approved, plasminogen activator called "TNK-tPA, which is easier and safer for clinicians to use. With a rapidly growing market, the question arises as to whether an opportunity exists to manufacture a generic form of tPA that can compete favorably with TNK-tPA.

In assessing the primitive problem, two promising alternatives might be generated by a design team:

Alternative 1. While a generic form of tPA may not compete well against TNK-tPA in the United States, it may be possible to market a low-cost generic tPA in foreign markets, where urokinase and streptokinase are low-cost alternatives, which sell for $200/dose, and are associated with increased bleeding risks. Market analysis suggests that a maximum production rate of 80 Kg/yr would be appropriate over the next five years.

Alternative 2. Given the possibility that lower healthcare reimbursements are received by hospitals in the United States, it may be reasonable to develop a similar process that competes favorably with TNK-tPA in the United States.

Other promising alternatives are likely to arise, often initiated by the successes of a research laboratory.

Note: Figure 1.1 has been revised to emphasize the key role of research in developing a good molecular structure and biochemical pathways to provide an effective drug. Emphasis is placed on laboratory testing and the need to develop a product that meets FDA approval and can be placed on the market rapidly. Since speed to market is dependent on the speed of process design, and since high-priced chemicals are involved, design optimization is normally not justified. However, it is crucial that process design take place in parallel with product research so that the pharmaceutical production can be scaled up rapidly (Pisano, 1997).

PRELIMINARY PROCESS SYNTHESIS (add following the vinyl chloride example in Section 2.4)

Example of Process Synthesis: Manufacture of Tissue Plasminogen Activator (tPA)

As introduced in Section 1.2, tissue plasminogen activator (tPA) is a recombinant therapeutic protein comprised of 562 amino acids, as shown schematically in Figure 1. Note that tPA is produced using a recombinant cell, which results from a recombination of genes. To eliminate blood clots, tPA activates plasminogen to plasmin, an enzyme, which dissolves fibrin formations that hold blood clots in place. In this way, blood flow is reestablished once the clot blockage dissolves; an important effect for patients with heart attack (microcardial infarction) or stroke. This example shows the steps in synthesizing a process to address the challenges posed by the specific problem posed in Alternative 1 of Section 1.2; that is, to manufacture less expensive forms of tPA that can be sold for $200 per 100 mg dose.

Stated differently, based upon extensive research in the biochemistry laboratory, the process synthesis problem in Figure 2 is created. As shown, tPA is to be produced, beginning with mammalian (e.g., Chinese hampster ovary (CHO)) cells that have tPA-DNA as part of their genetic contents (genome). The tPA gene was originally isolated from human melanoma cells. In an aerobic bio-reaction operation, the tPA-CHO cells grow in a nutrient media, HyQ PF-CHO – Hyclone media, a blend of nutrients, salts (including $NaHCO_3$), amino acids, insulin, growth factors, and transferrin, specifically for growth of CHO cells. Other ingredients include sterilized water, air, and CO_2. In addition to tPA, endotoxins may be a contaminant of the product, which must be removed because they elicit a variety of inflammatory responses in animals. Other by-products include cell debris, waste water, and gas emissions, especially N_2 from air, unconsumed O_2 from air, and CO_2, which regulates the pH. An important source of data, in addition to that taken in the biochemistry laboratory, is a U.S. patent, filed by Genentech (Goeddel, et al., 1988), which provides considerable qualitative and quantitative information.

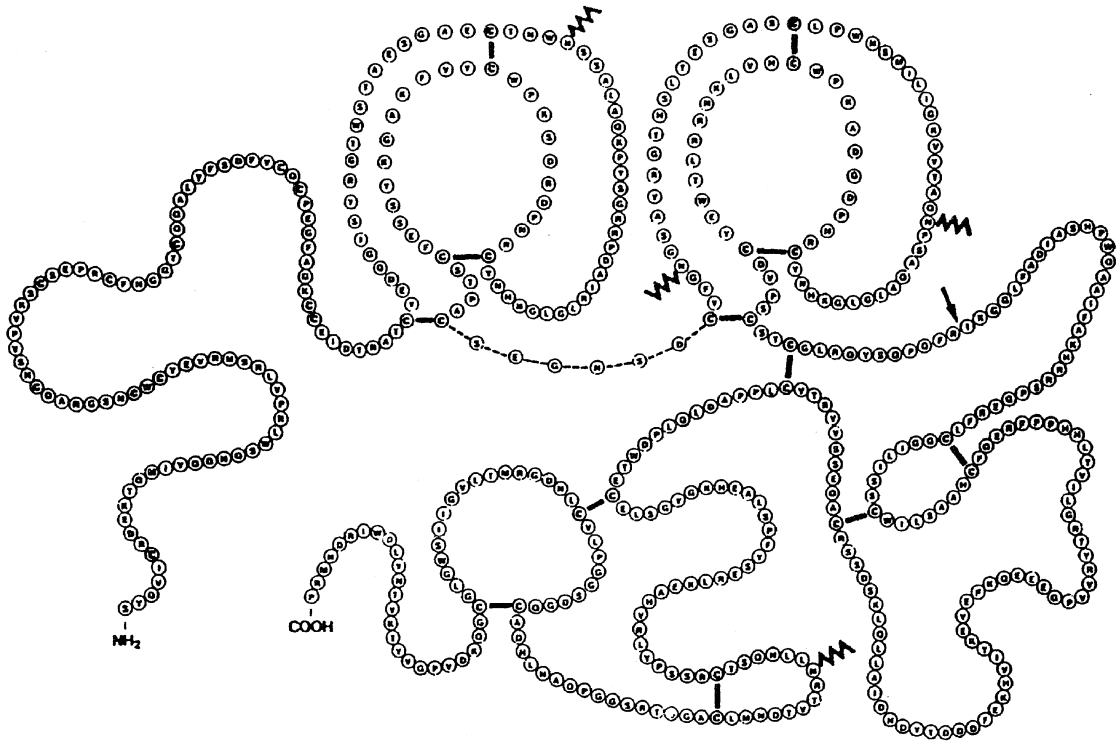


Figure 1. Schematic of tissue plasminogen activator (tPA)

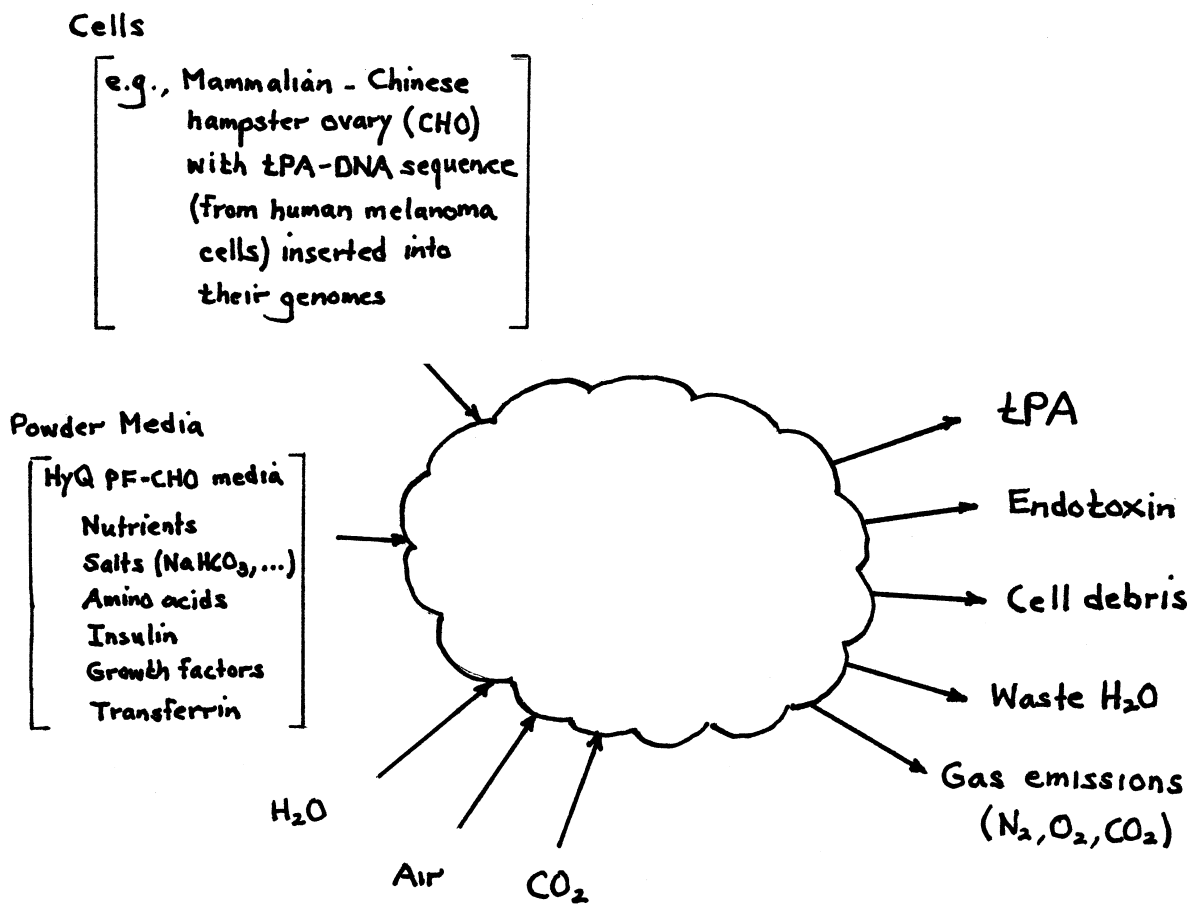


Figure 2. Process synthesis problem

Step 1. Eliminate Differences in Molecular Type

In the manufacture of a macro-molecule like tPA through cell growth, a complex array of chemical reactions is often approximated by global reactions that are understood far less than the well-defined reactions for the manufacture of a simple monomer, like vinyl chloride. In terms of global reactions to manufacture tPA, two principal reaction paths are provided by the biochemist, as follows.

1. Mammalian Cells

Into Chinese hamster ovary (CHO) cells, the tPA-DNA sequence must be inserted and expressed. The resulting tPA-CHO cells are specially selected CHO cells, with many copies of tPA-DNA inserted into their genomes, and secrete high levels of tPA. This tPA-DNA insertion step is summarized in the reaction:

$$\text{tPA-DNA sequence} + \text{CHO cells} \rightarrow \text{selected high expressing tPA-CHO cells} \quad (1)$$

The product of this "catalyst preparation" is a master stock of tPA-CHO cells, which are prepared in the laboratory and stored in 1-mL aliquots at -70°C to be used as inoculum for the bio-reaction:

$$\text{tPA-CHO cells} + \text{HyQ PF-CHO media} + O_2 \rightarrow \text{Increased cell numbers} \quad (2)$$

As the cells grow in this aerobic cultivation at a rate of 0.39×10^6 cell/(mL-day), oxygen from air is consumed at the rate of 0.2×10^{-12} mol O_2/(cell-hr), and tPA is produced at the rate of 50 pg tPA/(cell-day). The latter is secreted gradually into the liquid media solution. Note that reaction (1) is carried out once during the research and development phase. Initially, 1-10 mg of tPA-DNA are added to 10^6 cells to produce a few tPA-CHO cells in many unmodified CHO cells. After careful selection, one tPA-CHO cell (the "founder" cell) is selected and amplified to yield about 10^6 cells/mL in 10-100 L. These cells are frozen in aloquots.

2. Bacterial Cells

A promising alternative is to insert the tPA-DNA sequence into the genome of *Echerichia coli* (*E. coli*) cells, as summarized by the reaction:

tPA-DNA sequence + E. coli cells $\rightarrow$ selected high expressing tPA-E. coli cells (3)

Then, the tPA-E. coli cells, which are grown in the laboratory, are frozen in aliquots at -70°C to be used as inoculum for the fermentation reaction:

tPA-E. coli cells + powder media + O_2 $\rightarrow$ increased cell numbers (4)

A batch fermentation of tPA-E. coli can produce 5-50 mg tPA/L-broth at harvest. E. coli may require disruption to release tPA, which is then more difficult to separate. Should a process be synthesized based upon this reaction path, reaction rate data from the laboratory will be needed. Unlike CHO cells, E. coli cells do not add sugar groups (glycosylation) to tPA. Like CHO cells, tPA-E. coli cells are produced and frozen during the research and development phase.

Returning to the reaction path with CHO cells, using laboratory data, the reaction operation is inserted onto the flowsheet, as shown in Figure 3. At a production rate of 80 Kg/yr of tPA, the lab reports that the following ingredients are consumed and waste products are produced:

Ingredients		Wastes	
	Kg/yr		Kg/yr
tPA-CHO cells	small	Endotoxin	0.155
HyQ PF-CHO media	22,975	Cell debris	22,860
Water	178,250	Waste water	178,250
Air	3,740	Gas emissions (N_2, O_2, CO_2)	4,036
CO_2	296		

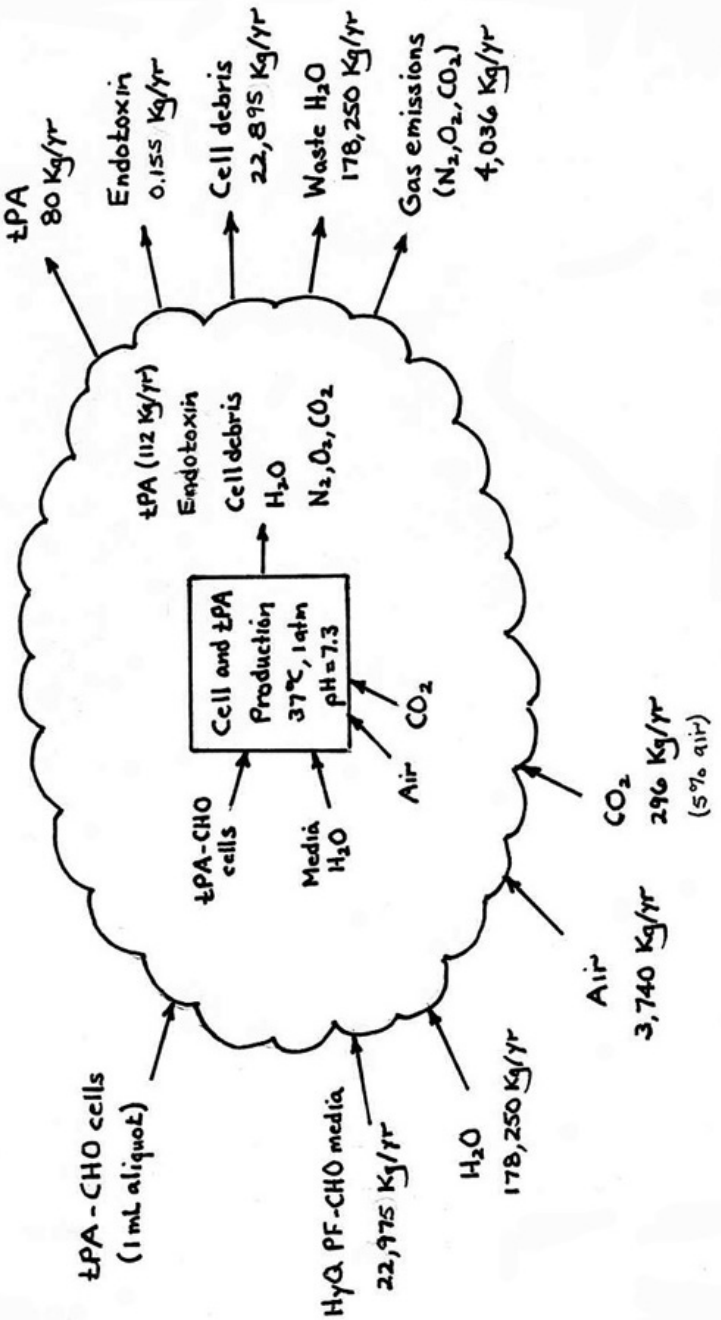


Figure 3. Reaction operations using mammalian CHO cells

The reaction operation provides sinks for tPA-CHO cells, from cold storage at -70°C, and HyQ PF-CHO media in water, air, and carbon dioxide. Its effluent is a source of tPA, at 112 Kg/yr, endotoxin, cell debris, water, nitrogen, and carbon dioxide. When separated, these species are the sources for the product sinks from the flowsheet. Note that the combined cell growth and tPA production operation takes place at 37°C, 1 atm, and pH = 7.3. The latter is achieved by the $NaHCO_3$ in the powder media, with fine tuning by manipulation of the flow rate of CO_2.

Before accepting a potential reaction path, it is important to examine the gross profit; that is, the difference between the sales revenues and the cost of ingredients. To accomplish this, the sales price of tPA is projected (e.g., $200 per 100 mg dose), and the costs of ingredients are projected, with estimates often obtained from the suppliers. A typical list of cost estimates is shown in Table 1. The cost of water for injection (WFI) is based upon estimates of the cost of sterilizing municipal water (12 cents/Kg = 45 cents/gal = $450/1,000 gal, which is far higher than the typical cost of process water = 50 cents/1,000 gal). The costs of sterilized air and carbon dioxide are for industrial cylinders of compressed gases. The cost of the tPA-CHO cells is not included, as it is associated with the cost of research, which is subsequently estimated as an operating cost.

Table 1 Assumed Cost of Chemicals Produced or Sold

Chemical	Kg/Kg tPA	Cost, $/Kg
tPA	1	2,000,000
HyQ PF CHO powder media	287.2	233
Water for injection (WFI)	2,228	0.12
Air	46.8	1,742
CO_2	3.7	1,447
tPA-CHO cells	-	*

* Not included in gross profit estimate – related to cost of research, an operating cost.

Using these costs, the gross profit is estimated:

$$\text{Gross Profit} = 2{,}000{,}000 - 287.2\times233 - 2{,}228\times0.12 - 3.7\times1{,}447 - 46.8\times1{,}742$$
$$= \$1{,}846{,}000/\text{Kg tPA}$$

Clearly, this is very high for tPA, a typical pharmaceutical. However, the gross profit does not account for the operating costs, which include the cost of research, the cost of utilities, and the investment cost, and are high for separations that involve expensive mass separating agents. With such a promising gross profit, the process synthesis proceeds at an accelerated pace.

Step 2. Distribute the Chemicals

In this step, the sources and sinks for each species in Figure 3 are matched so that the total mass flow rate into the reaction operation equals the mass flow rate out. This often entails the introduction of mixing operations, as illustrated in the previous example for vinyl chloride. In this case, only one mixing operation is introduced, in which the HyQ PF-CHO powder media is mixed with water, as shown in Figure 4. Otherwise the sources and sinks are matched directly. However, the effluent from the cell growth, tPA production reactor must be separated before its species are matched with the product sinks.

Step 3. Eliminate Differences in Composition

For most distributions of chemicals, composition differences exist between streams to be separated and the sinks to which these species are sent. Clearly, in Figure 4, the effluent from the cell growth, tPA production reactor must be separated.

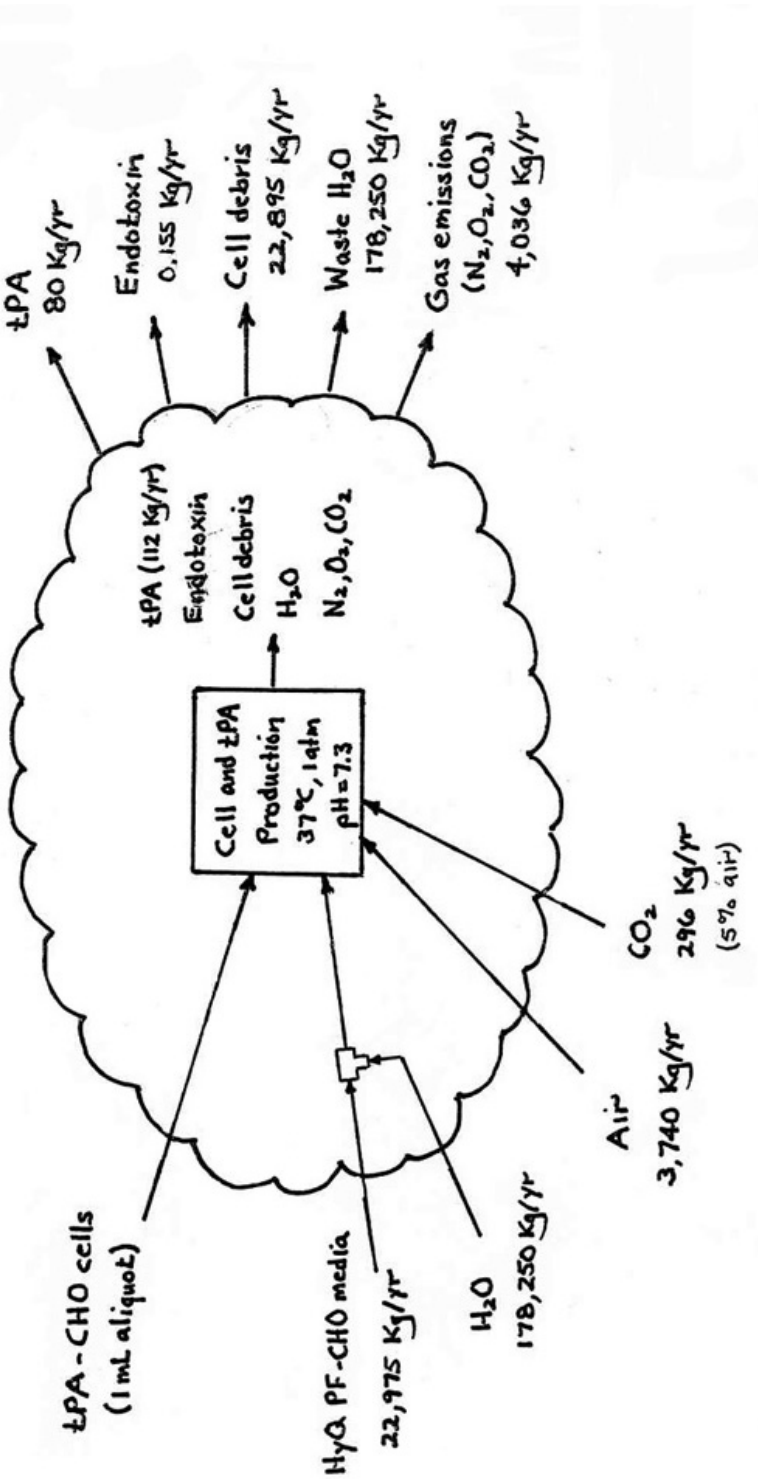
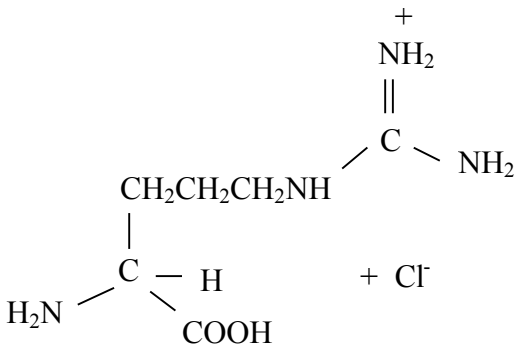


Figure 4. Flowsheet showing a distribution of chemicals for the tPA process

Many possibilities exist, with one provided in Figure 5. Here, the reactor effluent is sent to a separator for recovery of the gas emissions from the liquid mixture, with the latter sent to a centrifuge to remove wet cell debris from the harvest media or clarified broth. Note that because proteins lose their activity at temperatures above ~0°C, the centrifuge, and all other separation operations, are operated at 4°C, slightly above the freezing point of water. The harvest media is mixed with arginine hydrochloride, an amino acid:



which prevents tPA from self-aggregating. Note that 45,870 Kg/yr provides a concentration of 2.0 molar, which is sufficient to prevent aggregation.

The resulting mixture is sent to microfilters to remove large quantities of waste water, which passes through the filters. For this step, alternate separators, like gel filtration and an Acticlean Etox resin (by Sterogene) should be considered. The retentate from the filter, which contains tPA, other proteins, endotoxin, arginine hydrochloride, and some water, is sent to an affinity chromatography operation. Here, tPA is selectively adsorbed on a resin (e.g., CNBr-activated Sepharose, by Amersham Biotech). The resin is then eluted with glycine, an amino acetic acid:

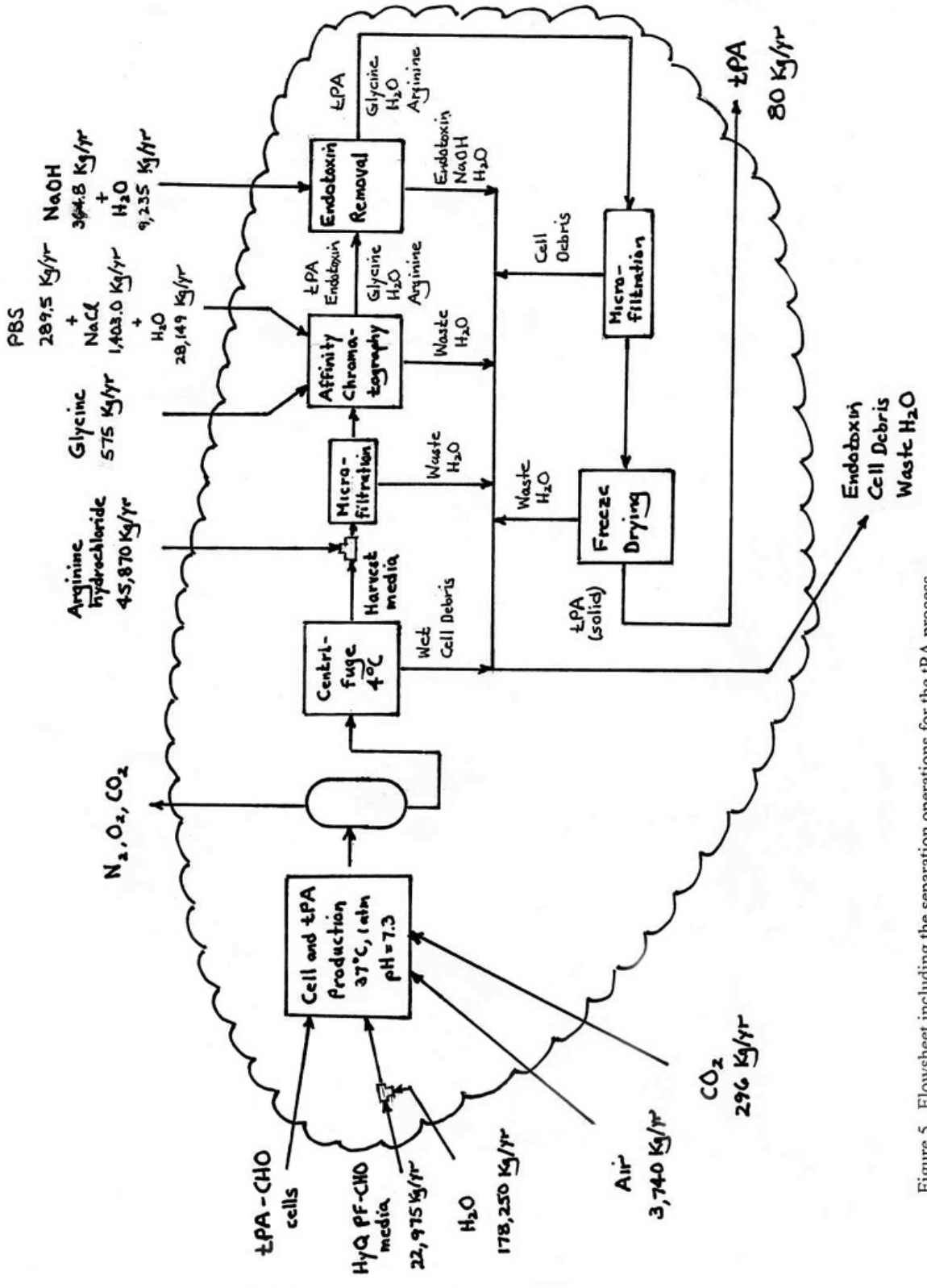
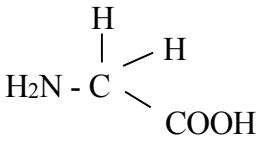


Figure 5. Flowsheet including the separation operations for the tPA process



From lab measurements, 575 Kg/yr of glycine are sufficient for the elution process. After the column is eluted, it is equilibrated with a mixture of 289.5 Kg/yr of phosphate buffer solution (PBS) and 1,403.0 Kg/yr of NaCl, with the quantities determined in the lab.

The resulting tPA solution is sent to an endotoxin removal column where the endotoxin is adsorbed selectively onto a resin (e.g., Acticlean Etox by Sterogene). This column is washed with a mixture of 364.8 Kg/yr of NaOH and 9,235 Kg/yr of water to remove the endotoxin. The effluent stream is microfiltered, to remove cell debris, which does not pass through the filter. Then, waste water is removed in a spray drying operation to provide tPA in powder form.

Step 4. Eliminate Differences in Temperature, Pressure, and Phase

In the manufacture of tPA, the ingredients are assumed to be available at 20°C, water is mixed with the HyQ PF-CHO powder media at 4°C, the cultivations (cell production operations) occur at 37°C, and the separations occur at 4°C. The exothermic heat of the cultivations is removed at 37°C. Only small pressure changes occur and can be neglected at this stage of process synthesis. Similarly, no phase-change operations are added to the flowsheet. Hence, only a few temperature change operations are added to Figure 5, with the resulting flowsheet shown in Figure 6.

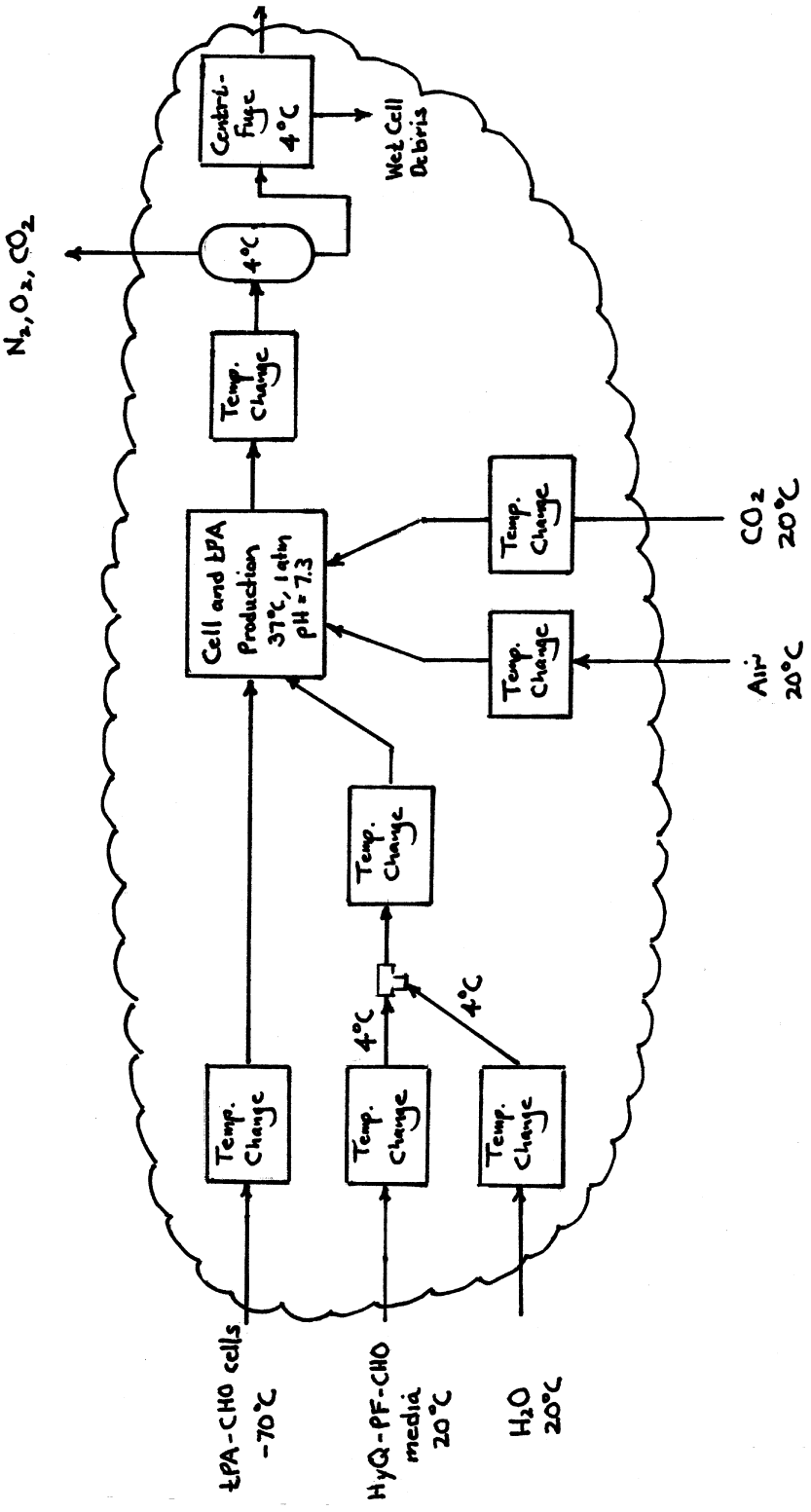


Figure 6. Flowsheet with the temperature-change operations in the tPA process

Step 5. Task Integration

At this stage in the synthesis, various items of equipment are selected, often combining two or more adjacent operations into a single equipment item; that is, *task integrating*. The first key decision involves whether to operate in continuous or batch mode. For small throughputs, such as 80 Kg/yr of tPA, the decision is nearly always to operate in batch mode. Choices of batch size and time are usually based upon the slowest operation, usually the cultivation process. For tPA, it is determined by the growth rate of tPA-CHO cells (0.39×10^6 cell/(ml-day)), the inlet and outlet concentrations (0.225×10^6 and 3×10^6 cell/mL), and the rate of tPA growth (50 pg tPA/(cell-day)). To produce 1.6 Kg of tPA per batch, 2.24 Kg of tPA are produced by cultivation, allowing for losses in the separation process. At this production rate, 2.24 Kg of tPA can be produced in 8 days in a 4,000 L batch (within a 5,000 L vessel). Allowing time for charging and cleaning, 14 days are reserved, and hence, 25 batches are produced annually, assuming 50 operating weeks. With two batch trains in parallel, 50 batches are produced annually; that is, 1.6 Kg of tPA are produced per batch.

The flowsheet in Figure 7a begins in a 1-L laboratory cultivator, into which a 1-mL aliquot of tPA-CHO cells is charged from cold storage at -70°C. To this, HyQ PF-CHO media, water, air, and CO_2 are added. Cultivation takes place over five days to produce 1.2 Kg/batch of inoculum, which is emptied from the cultivator and transferred to the plant in one day. This effluent inoculates three cultivators in series which carry out the cell and tPA production operation. The first is 40 L, with a 30 L batch that grows cells from 1.05×10^6 to 3×10^6 cell/mL in five days, with two additional days for loading and cleaning. The second is 400 L, with a 300 L batch that grows cells from 0.25×10^6 to 3×10^6 cell/mL in seven days, with 2.5 additional days for loading and cleaning. Finally, the third is 5,000 L with a 4,000 L batch that grows cells from 0.225×10^6 to 3×10^6 cell/mL in eight days, with six additional days for loading and cleaning. Note that gas emissions, containing N_2, O_2, and CO_2, are vented continuously from the cultivators. A 5,000 L mixing tank is installed to load and mix the powder media and water in two days.

Note the tank jacket through which refrigerant is circulated. This vessel is followed by a microfilter, which sterilizes the mixture by removing bacteria, and a hot water heat exchanger. One last vessel, a 5,000 L holding tank is provided to hold the contents of one cultivator batch (2.24, 457.17, 0.0031, 3,565 Kg/batch of tPA, tPA-CHO cells, endotoxin, and water), in the event that the centrifuge is taken off-line for servicing. The effluent from the third cultivator is cooled to 4°C in the shell-and-tube heat exchanger, which is cooled by a refrigerant on the shell side.

Turning next to the separation section in Figure 7b. The centrifuge is designed to handle small batches, at a rate of 400 L/hr over 10 hours. It rotates at high speed with the wet cell mass (which contains all of the tPA-CHO cells, five and 20 wt% of the tPA and water, and none of the endotoxin fed to the centrifuge) thrown to the outside collection volume and removed. Note that at this stage in process synthesis recovery fractions are estimated using heuristics and experimental data when available. Also, since the endotoxin contaminant must be removed entirely, it is assumed to be entirely recovered (100 percent) in the effluent from the microfilters. The clarified broth (2,854 Kg/batch) exits through the central tube overhead. It enters a mixing tank in which arginine hydrochloride is added to form a 1.1 molar solution, which is microfiltered to remove 3,494 Kg/batch of wastewater. The concentrated product, at 207 L/batch and containing 98, 5.62, and 5.62 wt% of the tPA, arginine hydrochloride, and water fed to the microfilter, is mixed with 67.4 Kg/batch of arginine in a second mixing vessel to give 2.0 molar arginine. This solution is microfiltered to remove particulate matter before being sent to the affinity holding tank. The effluent, which contains 95, 98, 100, and 98 wt% of the tPA, arginine, endotoxin, and water fed to the microfilter, is loaded into a 58 L affinity chromatography column, which adsorbs 100, 100, 2, and 2 wt% of tPA, endotoxin, arginine, and water is shown in Figure 7c. Most of the adsorbed tPA, 1.69 Kg/batch, is eluted with a stream containing glycine (523 Kg/batch at 2.2, 43.5, and 54.3 wt% of glycine, arginine, and water) and sent to a 500 L holding tank (405.7 Kg/batch containing 1.69, 8.7, 175.6, 0.0026, and 219.7 Kg/batch of tPA, glycine, arginine, endotoxin, and water.) Note that the elution buffer recovers 85 wt% of the tPA and endotoxin from the resin. The affinity chromatography column is equilibrated with an

equilibration buffer (597 Kg/batch containing 0.97, 4.7, and 94.3 wt% PBS, NaCl, and water). After a caustic and sucrose mix is added to the holding tank (0.013, 0.026, and 0.33 Kg/batch of NaOH, sucrose, and NaCl) the mixture is loaded into the endotoxin removal column (406.0 Kg/batch). In this 15.7 L column, the endotoxins are adsorbed, and removed, by washing with caustic (192 Kg/batch containing 3.8 and 96.2 wt% NaOH and water), which is discarded. The endotoxin removal column is regenerated with 47.1 Kg/batch of water while the endotoxin free solution (405.9 Kg/batch containing 1.6, 8.7, 175.6, 0.013, 0.026, 0.23, and 219.7 Kg/batch of tPA, glycine, arginine, NaOH, sucrose, NaCl, and water) is sent to a holding tank, where 59 Kg/batch of water are added. After sterilization with a microfilter to remove cell debris, from which 99.7 percent of the tPA is recovered, the solution is sent to a bottler and 100 mL vials, each containing 100 mg of tPA, are conveyed to a freeze drier, where the water is evaporated.

It is important to recognize that the batch sizes in Figure 7a,b,c are representative. However, as discussed subsequently in Chapters 4 and 12, the batch times and vessel sizes are key design variables in scheduling and optimizing batch processes.

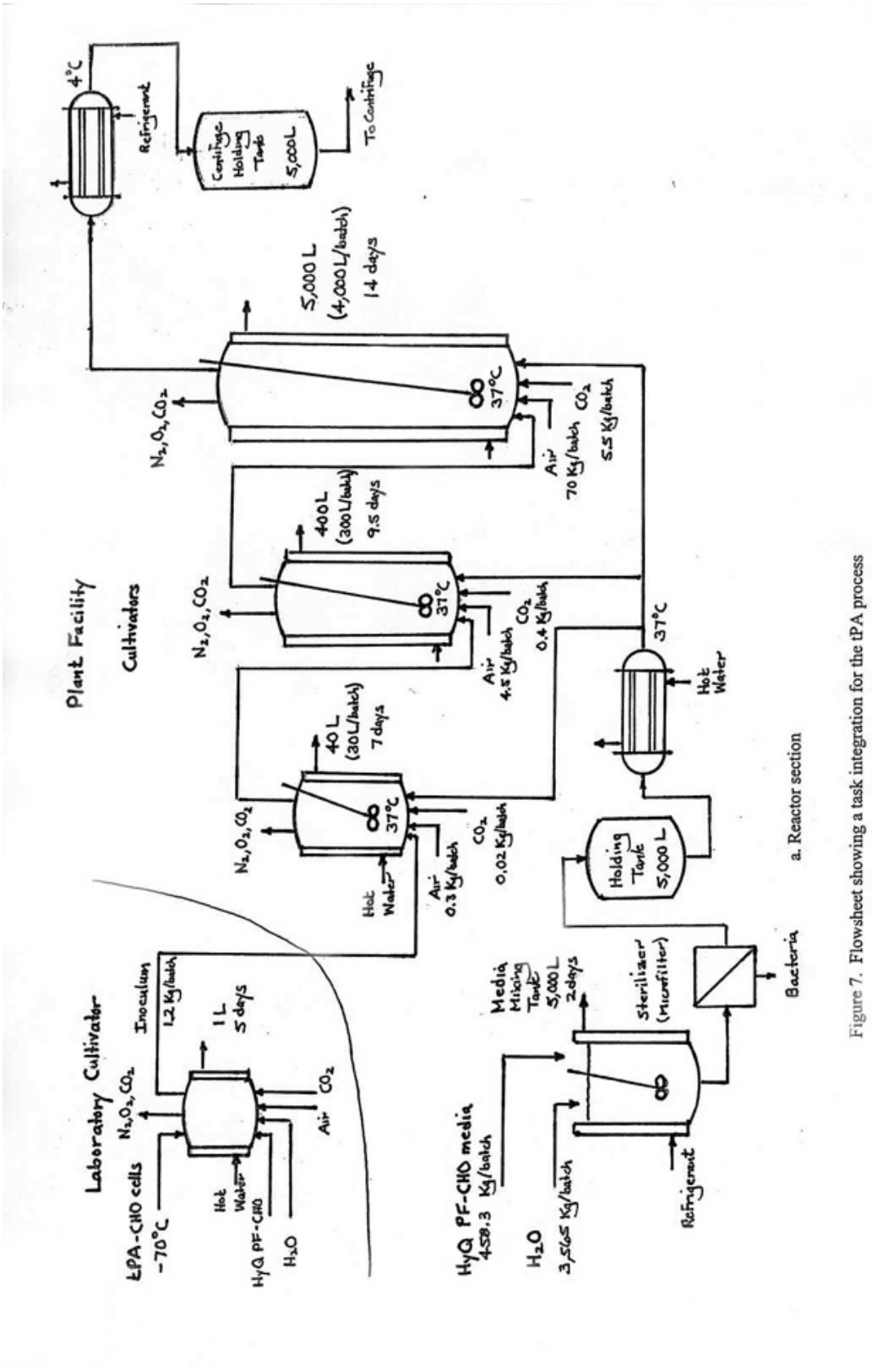
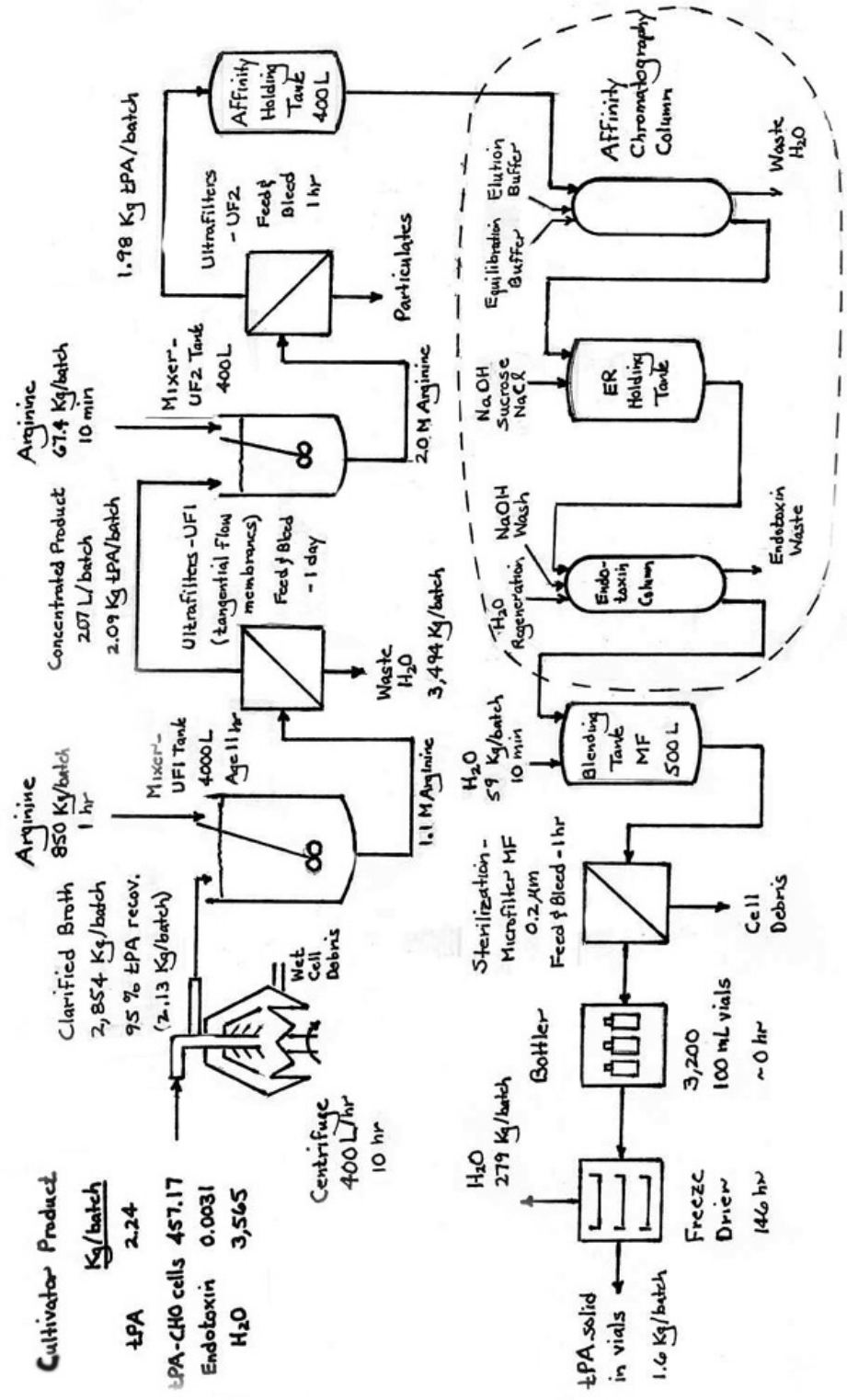
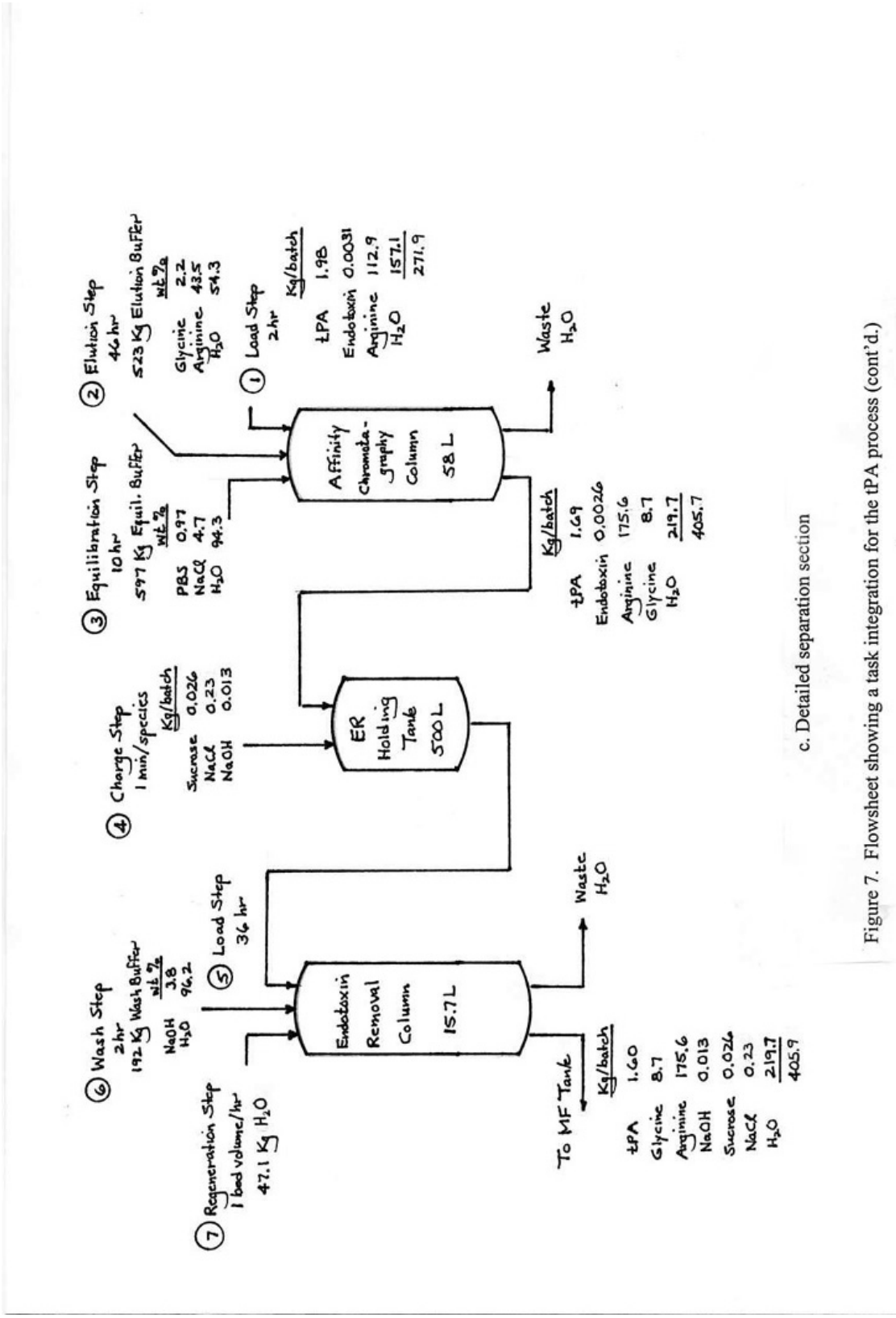


Figure 7. Flowsheet showing a task integration for the tPA process



b. Separation section

Figure 7. Flowsheet showing a task integration for the tPA process (cont'd.)



c. Detailed separation section

Figure 7. Flowsheet showing a task integration for the tPA process (cont'd.)

Synthesis Tree

Clearly, at each step in the synthesis of the process flowsheet, alternatives are generated and the synthesis tree fills in. For the tPA process, a schematic of a synthesis tree is shown in Figure 8. Note that the branch that corresponds to Figures 3-7 is displayed in boldface. In design synthesis, the engineer strives to identify the most promising alternatives, eliminating the least promising alternatives by inspection, wherever possible. Initially, heuristic rules help to make selections. Eventually, algorithm methods involving optimization are introduced to check the heuristics and identify more promising alternatives, as discussed in Chapter 12.

REFERENCES (to be added on page 63)

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Goeddel, D. V., Kohr, W. J., Pennica, D., and G. A. Vehar, *Human Tissue Plasminogen Activator*, U. S. Patent 4,766,075, August 23, 1988.

Pisano, G. P., *The Development Factory: Unlocking the Potential of Process Innovation,"* Harvard Business School Press, Boston, 1997.

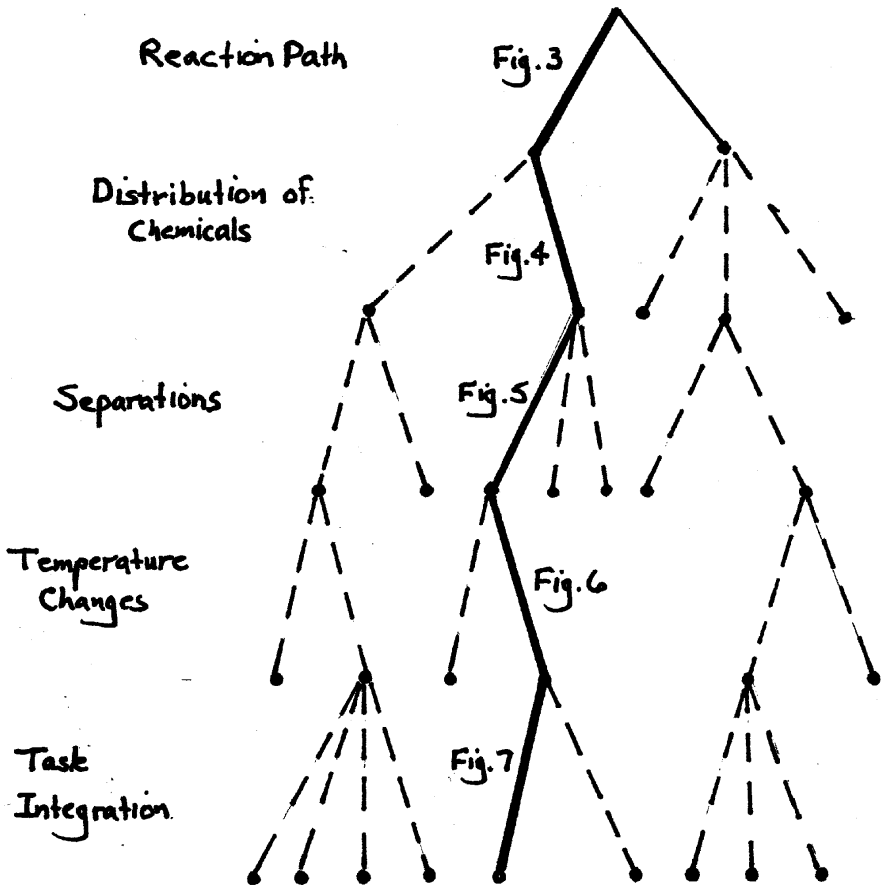

Figure 8. Inverted synthesis tree for the production of tPA

PRINCIPLES OF BATCH PROCESS SIMULATION

Supplement to Chapter 3 of
Process Design Principles: Synthesis, Analysis, and Evaluation

This supplement is the new Section 3.5. It was prepared with the assistance of Matt Fucci, who developed Example 1 and Exercises 1 and 2.

June 18, 2002
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3.5 PRINCIPLES OF BATCH FLOWSHEET SIMULATION

During the task integration step of process synthesis, as equipment items are selected, key decisions are made regarding whether they operate in continuous, batch, or semi-continuous modes, as discussed in Section 2.4. These decisions are based upon throughput and flexibility. When the throughput is small, for example, on the laboratory scale, continuous operation is often difficult and impractical to maintain, it usually being simpler and more profitable to complete a batch in hours, days, or weeks. Even for larger throughputs, where multiple products are produced, with variably-sized orders received regularly, batch processes offer the ease of switching from the production of one product to another; that is, flexibility, which is more difficult to achieve in continuous operation.

As shown for the manufacture of tissue plasminogen activator (tPA) in Section 2.4, when batch operation is selected for an equipment item, either batch time or size must be selected, with the other determined as a function of the throughput specification (e.g., 80 Kg/yr of tPA). Furthermore, when the product throughput is specified, the throughput through each process unit is determined, as shown in the synthesis of the tPA process in Section 2.4. In many cases, available vessel sizes are used to determine the size of a batch and, in turn, the batch time.

Given the process flowsheet and the specifics of operation for each equipment item, it is the role of batch process simulators, like BATCH PLUS™, by Aspen Tech, and SUPERPRO DESIGNER™, by Intelligen, Inc., to carry out material and energy balances, and to prepare an operating schedule; that is, a Gantt chart for the process. Then, after the equipment and operating costs are estimated, and profitability measures are computed, the batch operating parameters and procedures can be varied to increase the profitability of the design.

Process and Simulation Flowsheets

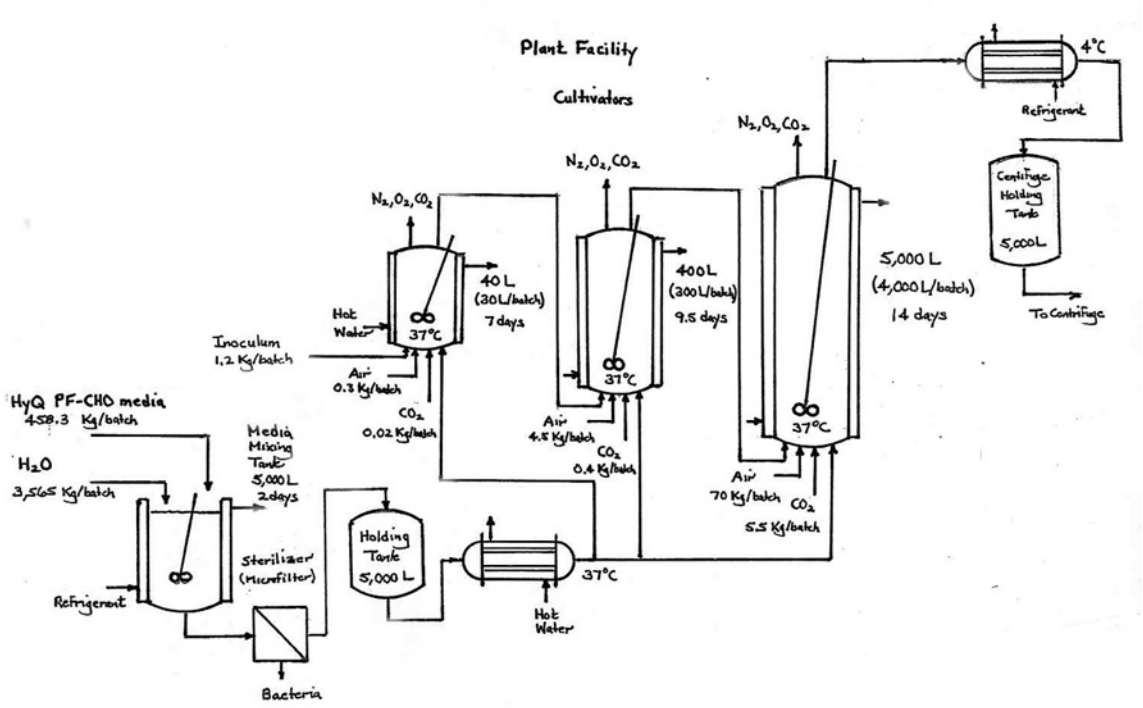
Like in the steady-state simulation of continuous processes, it is convenient to convert from a *process flowsheet* to a *simulation flowsheet*. To accomplish this, it is helpful to be familiar with the library of models (or *procedures* or *operations*) provided by the simulator. For example, when using SUPERPRO DESIGNER to simulate two fermentation reactors in series, the process flowsheet in Figure 1a is replaced by the simulation flowsheet in Figure 1b. In BATCH PLUS, however, this conversion is accomplished without drawing the simulation flowsheet, since the latter is generated automatically on the basis of the recipe specifications for each equipment item.

In the simulation flowsheets, the arcs represent the streams that convey the batches from equipment item to equipment item. Each arc bares the stream name and represents the transfer of information associated with each stream; that is, the mass of each species per batch, temperature, pressure, density, and other physical properties.

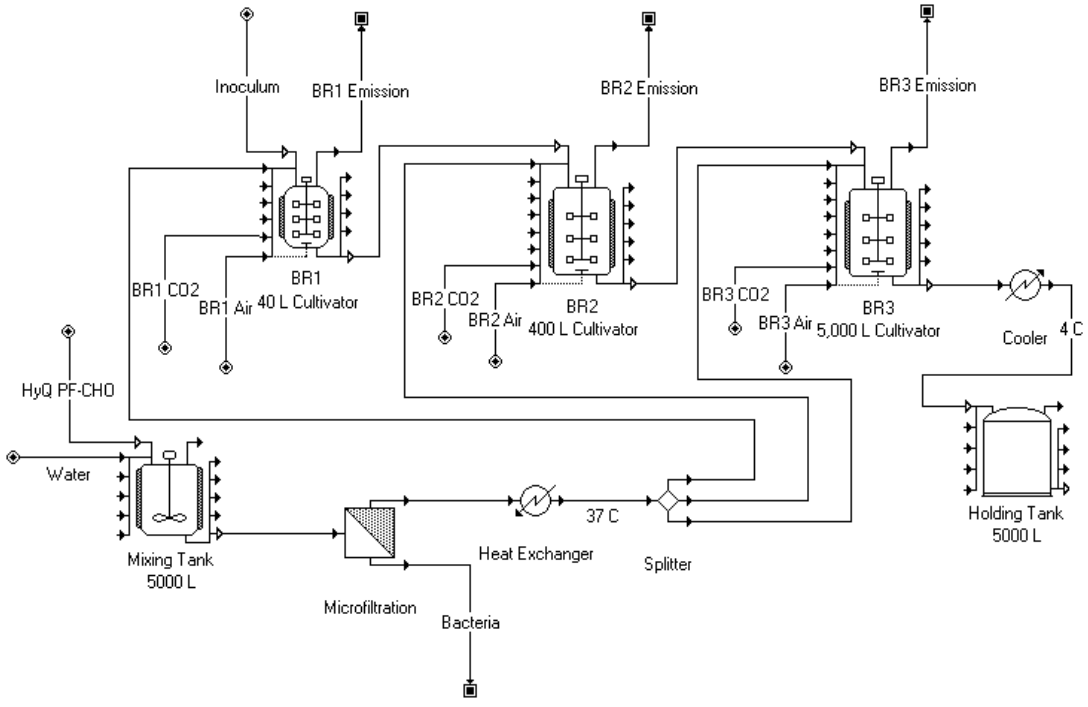
The icons represent the models for each of the equipment items. Unlike for the simulation of processes in the steady state, these models involve a sequence of process operations which are specified by the designer. Typically, these operations are defined as a *recipe* for each equipment item, and usually involve charging the chemicals into the vessel, processing the chemicals, removing the chemicals from the vessel, and cleaning the vessel. Note that in the SUPERPRO DESIGNER simulation flowsheet in Figure 1b, the microfiltration model represents both the microfilter and its holding tank in the process flowsheet, Figure 1a.

Equipment Models

Table 1 lists the equipment models (or procedures or operations) in each of the two simulators. Some of the models carry out simple material balances given



(a) Process flowsheet



(b) SUPERPRO DESIGNER simulation flowsheet

Figure 1. Flowsheets

Table 1 Equipment Models

(a) BATCH PLUS Equipment Models

Class	Mode	Type
Adsorption	Batch	Adsorption System
Agitator	Continuous	Agitator - 3-Blade Retreat Impeller, Helical Ribbon, Paddle, Propeller, Turbine
Biotech	Batch	Autoclave, Cell Factory, Diafilter, Filter-Depth, Incubator, Incubator-shaker, Laminar Flow Hood, Lyophilizer, Microfilter, Triblender, Ultrafilter
	Continuous	Bead Mill, Homogenizer, Sterilizer, Transfer Panel, Valve
Centrifuge	Batch	Centrifuge, Centrifuge - Decanter, Disk-Stack, Filter, Horizontal Basket, Multichamber-Bowl, Tubular-Bowl, Vertical Basket
Column	Batch	Column, Column-Chromatography
	Continuous	Column - Continuous Packed, Continuous Tray
Compressor	Continuous	Compressor, Blower, Fan
Conveyor	Continuous	Conveyor-Pneumatic
Crystallizer	Batch	Crystallizer
	Continuous	Crystallizer-Continuous
Dryer	Batch	Dryer, Dryer - Agitated Pan, Blender, Conical, Freeze, Fluid Bed, Horizontal Paddle, Rotary, Spray, Tray
	Continuous	Dryer-Continuous, Fluid Bed-Continuous
Emission Control	Either	Vapor Emission Vent
Evaporator	Continuous	Evaporator - Long Tube, Thin Film, Wiped Film
Extractor	Batch	Extractor
	Continuous	Extractor – Continuous
Fermentor	Batch	Fermentor
	Continuous	Fermentor – Continuous
Filling	Continuous	Filling System
Filter	Batch	Filter – Agitated Nutsche, Air, Bag, Belt, Cross Flow, Dryer, In-line, Pot, Press, Sparkler, Tank Sheet
	Continuous	Filter – Continuous
Formulation and Packaging	Batch	Blender, Coater, High Gear Granulator, Kneader, Mill-Hammer, Screen, Sifter
	Continuous	Classifier, Extruder, Filling System, Granulator-Fluid Bed, Mill – Continuous, Jet; Tableting Unit

Table 1 Equipment Models (Cont'd.)

(a) BATCH PLUS Equipment (Cont'd.)

Class	Mode	Type
Changing Component for Formulation	Continuous	Air Distributor Plate, Agitator – Impeller, Blade; Chopper, Distribute Plate, Filter Socks, Nozzle, Screen – Mill
Generic	Batch	Generic Batch
Heat Exchanger	Batch	Condenser
	Continuous	Cooling Tower, Electric Heater, Fired Heater, Heat Exchanger, Heat Exchanger Plate, Heat Exchanger Shell and Tube, Refrigeration Unit
Heat Transfer	Batch	Internal Helical-Coil, Jacket – Agitated Conventional, Baffled Conventional, Conventional, Dimple, Half-pipe Coils
Hopper	Batch	Hopper, Plate Feeder
Instrument		Flow Meter, Moisture Analyzer, Scale, Tester – Hardness, Friability, Thickness; Disintegration Bath
Mixer	Batch	Mixer
	Continuous	Mixer – In-Line
Piping	Batch	Piping
Pump	Continuous	Pump, Pump – Liquid Ring Vacuum, Vacuum
Reactor	Batch	Reactor
	Continuous	Reactor – Continuous
Scrubber	Batch	
Solid Transport	Continuous	Screw Conveyor, Vacuum-Pressure Lock
Storage Location	Batch	Inventory Location, Inventory Location-Vapor
Tank	Batch	Tank
Miscellaneous	Continuous	After Burner, Cyclone, Demister, Dust Collector, Ejector, Hydrocyclone, Steam Jet

Table 1 Equipment Models (Cont'd.)

(b) BATCH PLUS Operations

Batch Operations	Age, Centrifuge, Charge, Clean, Cool, Concentrate, Crystallize, Decant, Distill, Dry, Evacuate, Extract, Filter, Filter-in-Place, Heat, Heat-to-Reflux-and-Age, Line-Blow, Line-Flush, Open/Close-Vent, pH-Adjust, Pressurize, Purge, QC-Test, Quench, Quench-in-Place, React, React-Distill, Start-Sweep, Stop-Sweep, Transfer, Transfer-Through-Heat-Exchanger, Utilize, Vent, Wash-Cake, Yield-React
Chromatography Operations	Elute-Column, Equilibrate-Column, Load-Column, Regenerate-Column, Wash-Column
Continuous Operations	Crystallize-Continuously, Distill-Continuously, Dry-Continuously, Extract-Continuously, Filter-Continuously, React-Continuously
Biotech Operations	Cell-Disrupt, Centrifuge-By-Settling, Depth-Filter, Diafilter, Ferment, Ferment-Continuously, Microfilter, Sterilize, Transfer-Through-Sterilizer, Ultrafilter

(c) SUPERPRO DESIGNER Procedures (Equipment Models)

Group	Mode	Type
Vessel	Batch	Reactor, Fermentor, Seed Fermentor, Airlift Fermentor
Continuous Reaction	Continuous	Stoichiometric (CSTR, PFR, Fermentor, Seed Fermentor, Airlift Fermentor)
	Continuous	Kinetics (CSTR, PFR, Fermentor, Seed Fermentor)
	Continuous	Equilibrium (CSTR)
	Continuous	Environmental (Well-mixed Aerobic, BioOxidation, …
Filtration	Batch	Microfiltration, Ultrafiltration, Reverse Osmosis, Diafiltration, Dead End Filtration, Nutsche Filtration, Plate & Frame Filtration, Baghouse Filtration, Electrostatic Precipitation
	Feed and Bleed (continuous)	Microfiltration, Ultrafiltration, Reverse Osmosis
	Either	Rotary Vacuum Filtration, Air Filtration, Belt Filtration, Granular Media Filtration, Baghouse Filtration, Electrostatic Precipitation
Centrifugation	Batch	Basket Centrifuge
	Either	Decanter Centrifuge, Disk-stack Centrifuge, Bowl Centrifuge, Centritech Centrifuge, Cyclone, Hydroclone
Homogenization	Either	High Pressure, Bead Milling
Chromatography/ Adsorption	Batch	Gel Filtration, Packed Bed Adsorption (PBA) Chromatography, Granular Activated Carbon (GAC) – liquid and gaseous stream

Table 1 Equipment Models (Cont'd.)

(c) SUPERPRO DESIGNER Procedures (Cont'd.)

Group	Mode	Type
Drying	Batch	Tray Drying, Freeze Drying
	Either	Spray Drying, Fluid Bed Drying, Drum Drying, Rotary Drying, Sludge Drying
Sedimentation	Either	Decanting (2-liquid phases), Clarification, Inclined Plane (IP) Clarification, Thickiner Basin, Dissolved Air Flotation Tank, Oil Separator
Distillation	Batch	Shortcut Batch Distillation
	Either	Flash Drum, Shortcut Distillation
Extraction	Either	Mixer-Settler, Differential Column Extractor, Centrifugal Extractor
Phase Change	Either	Condensation for Gas Streams, Multiple-effect Evaporation, Crystallization
Adsorption/Stripping	Either	Absorber, Stripper, Degasifier
Storage	Batch	Hopper, Equilization Tank, Junction Box Mixing
	Either	Blending Tank, Flat Bottom Tank, Receiver, Horizontal Tank, Vertical On Legs Tank, Silo
Heat Exchange	Either	Heating, Electrical Heating, Cooling, Heat Exchanging (2-streams), Heat Sterilization
Mixing	Batch	Bulk Flow (Tumble Mixer)
	Either	Bulk Flow (2-9 streams), Discrete Flow (2-9 streams)
Splittiing	Either	Bulk Flow (2-9 streams), Discrete Flow (2-9 streams), Component Flow (2-9 streams)
Size Reduction	Either	Grinding (Bulk or Discrete Flow), Shredding (Bulk or Discrete Flow)
Formulation & Packaging	Either	Extrusion, Blow Molding, Injection Molding, Trimming, Filling, Assembly, Printing, Labeling, Boxing, Tableting
Transport (near)	Either	Liquid (Pump) Gas (Compressor, Fan) Solids (Belt Conveyor – bulk or discrete flow, Pneumatic Conveyor - bulk or discrete flow, Screw Conveyor - bulk or discrete flow, Bucket Elevator - bulk or discrete flow)
Transport (far)	Either	By Land (Truck - bulk or discrete flow, Train - bulk or discrete flow) By Sea (Ship - bulk or discrete flow) By Air (Airplane - bulk or discrete flow)

Table 1 Equipment Models (Cont'd.)

(d) SUPERPRO DESIGNER Operations

Absorb	Adsorb	Agitate	Assemble
Bio-oxidize	Bio-react	Centrifuge	Charge
Clarify	Clean-in-place (CIP)	Compress	Concentrate (Batch)
Concentrate (Feed & Bleed)	Condense	Convert to Bulk	Convert to Discrete
Convey	Cool	Crystallize	Cyclone
Cycloning	Decant	Degasify	Diafilter
Distill	Dry	Dry Cake	Elevate
Elute	Equalize	Equilibrate	Evacuate
Exchange Heat	Extract/Phase Split	Extrude	Ferment (Kinetic)
Ferment (Stoichiometric)	Fill	Filter	Flash
Flotate	Gas Sweep	Grind	Handle Solids Flow
Heat	Hold	Homogenize	Incinerate
Label	Load	Mix	Mix Solids
Mold	Neutralize	Oxidize	Pack
Pass Trough	Precipitate	Pressurize	Print
Pump	Pump Gas	Purge/Inlet	Radiate
React (Equilibrium)	React (Kinetic)	React (Stoichiometric)	Regenerate
Separate Oil	Shred	Split	Steam-in-place (SIP)
Sterilize	Store	Store Solids	Strip
Tablet	Thicken	Transfer In	Transfer Out
Transport	Trim	Vaporize/Concentrate	Vent
Wash	Wash Cake		

specifications for the feed stream(s) and the batch (or vessel) size or batch time. Others, like the batch distillation models, integrate the dynamic MESH (Material balance, Equilibrium, Summation of mole fractions, Heat Balance) equations given specifications like the number of trays, the reflux ratio, and the batch time. Detailed documentation of the equipment models is provided in user manuals and help screens.

More specifically, a list of the BATCH PLUS Equipment Models is provided in Table 1a. These are organized under the *class* of model, with a list of *type* of equipment, and an indication of whether a model can be used in *batch*, *continuous*, or *either mode.* Similarly, for SUPERPRO DESIGNER, a list of *procedures* (equipment models) is provided in Table 1c. These are organized here as *groups* of equipment types.

For each equipment item, the engineer must specify the details of its operations. These include specifications for charging, processing, emptying, and cleaning. When using BATCH PLUS, these are specified in the steps in a recipe, with the equipment items defined as the steps are specified. A full list of the operations available is provided in Table 1b. Following this discussion, the results for a BATCH PLUS simulation of the reactor section of the tPA process are provided in Example 1, with step-by-step instructions provided for specifying the operations and equipment items provided in the last subsection of Section 3.5. In SUPERPRO DESIGNER, since the engineer provides a simulation flowsheet, the operations are specified unit-by-unit. Its list of operations is provided in Table 1d.

Batch and Continuous Operation

Since it is possible to have adjacent equipment items operating in batch and continuous modes, it is important to understand the conventions used when preparing a mixed simulation with batch and continuous operations. In most cases, it is desirable to install a holding tank to moderate the surges that would otherwise occur.

In SUPERPRO DESIGNER, each flowsheet is defined by the engineer as either *batch* or *continuous*. In batch mode, stream results are reported on a per batch basis, even for streams associated with continuous processes in a batch flowsheet. Each equipment item is designated as operating in batch/semi-continuous or continuous mode. Scheduling information must be included for all items designated as operating as batch/semi-continuous. Semi-continuous units operate continuously while utilized, but are shut down between uses. Equipment items designated as continuous are assumed to operate at all times, and are excluded from operation schedules (and Gantt charts).

When a SUPERPRO DESIGNER flowsheet is defined to be in continuous mode, streams are reported in a per hour basis. Scheduling information is not required, and no overall batch time is calculated. Individual batch processes can be inserted into the flowsheet, with their batch and turnaround times specified.

In BATCH PLUS, every simulation is for an overall batch process, with stream values always reported on a per batch basis. Continuous operations, however, can be inserted. For these units, a feed is loaded, the vessel is filled to its surge volume, and an effluent stream immediately begins to transfer the product downstream. This differs from normal batch operation, which involves loading all of the feed and completing the processing steps before unloading. Specific units in BATCH PLUS, such as the Fermenter, can also operate as fed-batch. In such operations, a feed is added continuously to the batch while an operation is taking place.

With SUPERPRO DESIGNER and BATCH PLUS, caution must be taken when introducing continuous operations into batch processes, as no warnings are provided when a continuous process unit is running dry. When a feed to a continuous unit runs dry, the simulator assumes that this unit is shut down and restarted when the feed returns. Clearly, such operation is infeasible for many units, such as distillation columns and chemical reactors. Consequently, when continuous processes are included, it is important to check the results computed by the batch simulators to be sure that unreasonable assumptions have not been made.

An advantage of adding continuous operations arises when the process bottleneck is transferred to the continuous unit. When a schedule is devised such that the continuous unit is always in operation, batch cycling is avoided.

Example 1 tPA Cultivators

tPA-CHO cells are used to produce tPA, as discussed in Section 2.4. These cells are duplicated to a density of 3.0×10^6 cell/mL, after which the culture becomes too dense and the tPA-CHO cells die at a high rate. For this reason, engineers cultivate tPA-CHO cells in a sequence of bioreactors, each building up mass to a density of 3.0×10^6 cell/mL, with the accumulated cell mass used to inoculate the next largest reactor, until the desired cell mass is reached.

In this example, the objective is to determine the effective time between batches; that is, the *batch time*, which is less than the total occupied time of a sequence of batch operations. The batch time is smaller because while one batch is moving through the sequence, other batches are being processed simultaneously in other pieces of equipment both upstream and downstream. Therefore, the effective time between batches, or the batch time, is determined by the equipment unit that requires the most processing time. This equipment unit is known as the *bottleneck*, and consequently, to reduce the batch time, engineers seek to reduce the processing time of the bottleneck as much as possible. Usually, the bottleneck is associated with the largest process unit, often the main bioreactor, because these reactors involve the largest cultivation times.

For this example, the BATCH PLUS simulator is used to determine the cycle time for a portion of the tPA process that involves just two cultivators, as shown in Figure 2. Initially, a mixing tank is charged with 3,565 Kg of water and 458.3 Kg of HyQ PF-CHO media, with a charge time of one hour. The material in the tank is cooled to 4°F for one day and aged for two days to allow for quality

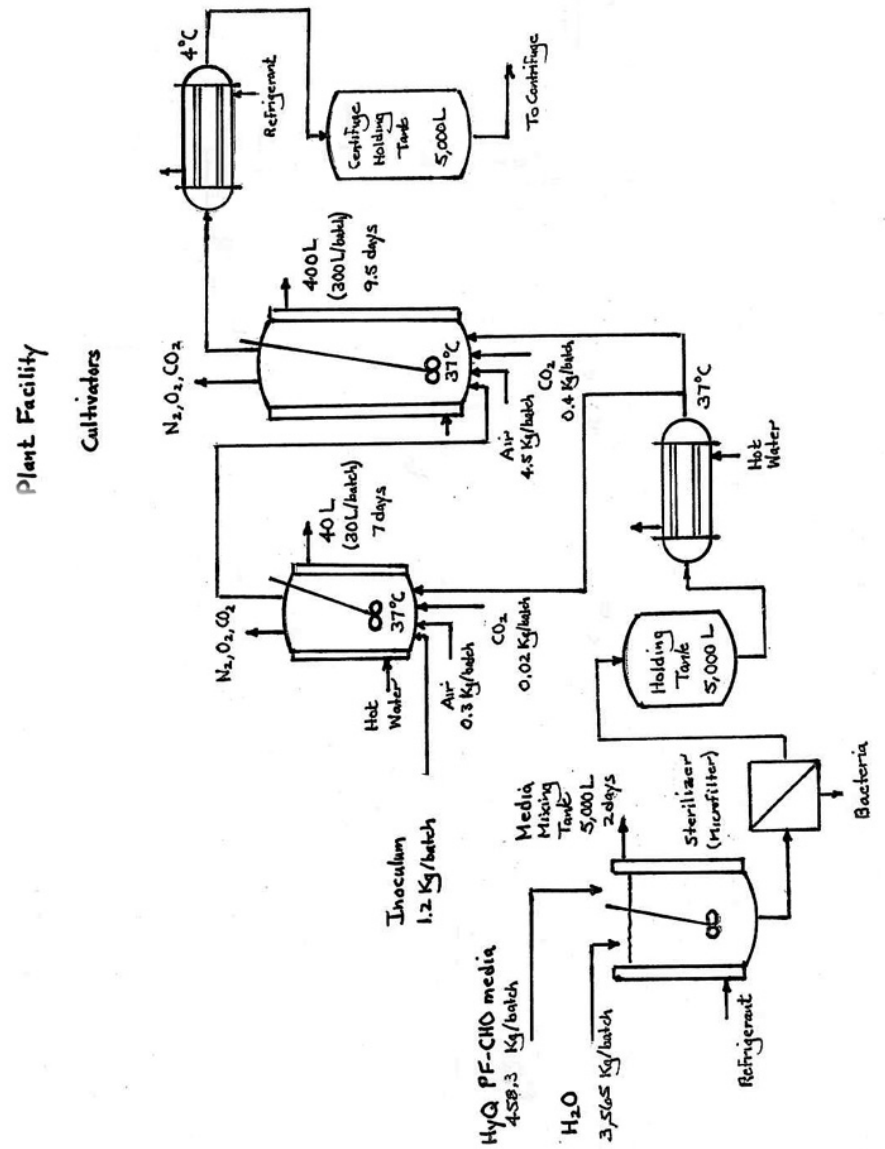


Figure 2. tPA reactor section with two cultivators

assurance testing. Then, this material is transferred to a 0.2 μm microfilter for sterilization, to remove bacteria over a two-hour period, and sent to a holding tank. Next, the first cultivator is charged with 1.2 Kg of tPA-CHO cells in one hour. Then, 21.2 Kg of material from the holding tank are heated in a heat exchanger to 37°F and added to the first cultivator in 0.5 day, after which cultivation takes place over the next five days. The yield from the cultivation is 15.3 wt% tPA-CHO cells, 0.01 wt% endotoxin, 84.7 wt% water, and 0.01 wt% tPA. The products of Cultivator 1 are fed to Cultivator 2 in 0.5 day. Then, 293.5 Kg of media from the holding tank are heated to 37°F and fed to Cultivator 2 in 0.5 day, after which the cultivation takes place over seven days. Immediately after Cultivator 1 is emptied, it is cleaned-in-place using 60 Kg of water over 20 hours. Note that to override the BATCH PLUS estimate, a charge time of 1 min should be entered. Then, it is sterilized at 130°C for two hours and cooled to 25°C (with one-hour heat-up and cool-down times). The yield of the cultivation in Cultivator 2 is 11.7 wt% tPA-CHO cells, 7.67×10^{-4} wt% endotoxin, 88.3 wt% water, and 0.039 wt% tPA. After this cultivation, the contents of Cultivator 2 are cooled in a heat exchanger to 4°C and transferred to the centrifuge holding tank over 0.5 day. After Cultivator 2 is emptied, it is cleaned-in-place using 600 Kg of water over 20 hours, and sterilized using the procedure for Cultivator 1.

To determine the cycle time and the bottleneck unit, create a multiple batch Gantt chart using BATCH PLUS. Generate equipment content and capacity reports to determine the sizes of the equipment items. Examine the stream table report to monitor the production of tPA-CHO cells and tPA in the process.

Solution:

When using BATCH PLUS, as discussed in the last subsection of Section 3.5, the materials are specified; that is, tPA-CHO cells, tPA, media, water, nitrogen, oxygen, and carbon dioxide. Then, each equipment item is entered with its recipe of operations. Note that there is no cultivator model in BATCH PLUS, and consequently, the *Fermenter* model is used in its place. Given this information, BATCH PLUS generates a recipe of operations for the process, shown in Figure 3a, and prepares the simulation flowsheet (using Microsoft VISIO™), shown in Figure

3b. BATCH PLUS also generates a table including the per batch flow rates of each stream in the process in a time-dependent manner, as shown in Figure 3c. Study of this report allows the monitoring of the growth of tPA-CHO cells, and their production of tPA, as they travel from vessel to vessel. The third column from the last in the report indicates that the final stream in the process contains 36.9 Kg of tPA-CHO cells, 0.12 Kg of tPA, 0.0024 Kg of endotoxin, and 278.8 Kg of water.

In addition, BATCH PLUS uses Microsoft EXCEL™ to prepare an *Equipment Contents Report*, which displays, for each vessel in the process, an inventory of the contents of the vessel during each step the vessel is utilized. This includes the mass of components, as well as overall liquid and solid volume and mass. Inspection of these reports allows estimation of required vessel sizes. The report for the Mixing Tank, shown in Figure 3d, indicates a maximum liquid and solid volume of 4,050 L after operation 1.1. It can, therefore, be concluded that the Mixing Tank unit must be larger than 4,050 L; that is, 5,000 L. Similarly, the reports for Fermenters 1 and 2, shown in Figures 3e and Figure 3f, indicate maximum volumes of 22.8 L and 322 L after operations 1.7 and 1.8. On this basis, 40 L and 400 L vessels are selected for Fermenters 1 and 2.

Finally, when the Gantt Chart prepared by BATCH PLUS is extended to show three batches, as shown in Figure 3g, the bottleneck of the process is determined quite easily. For each vessel, solid blocks show the time period during which it is in operation. Solid blocks in red, blue, and green are for the first, second, and third batches, respectively. The bottleneck is associated with the equipment unit that is utilized at all times; that is, for which the red, blue, and green blocks touch each other. Clearly, this unit determines the cycle time.

1.1. Charge Mixing Tank with 458.3 kg of Media. The charge time is 1 h. Charge Mixing Tank with 3565 kg of WATER. The charge time is 1 h.

1.2. Cool unit Mixing Tank to 4 C. The cooling time is 1 day.

1.3. Age the contents of unit Mixing Tank for 2 day.

1.4. Microfilter the contents of Mixing Tank in Microfilter. The mode of operation is Batch Concentration. Unspecified components go to the Permeate. The operation time is 2 h. The permeate stream is sent to Holding Tank.

1.5. Charge Fermenter 1 with 1.2 kg of tPA-CHO Cells. The charge time is 1 h.

1.6. Transfer contents of unit Holding Tank to Fermenter 1 through heat exchanger Heat Exchanger. The final stream temperature is 37 C. Transfer 21.2 kg of vessel contents. The transfer time is 0.5 day.

1.7. Ferment in unit Fermenter 1. The yield of tPA-CHO Cells in the Solid phase is 0.153, of Endotoxin in the Liquid phase is 0.0001, of tPA in the Liquid phase is 0.0001, of WATER in the Liquid phase is 0.847, of Media in the Liquid phase is 0, of Media in the Solid phase is 0 and of tPA-CHO Cells in the Liquid phase is 0. The fermentation time is 5 day. Continuously add 0.02 kg of CARBON-DIOXIDE. Continuously add 0.3 kg of AIR.

Start Parallel

Series

1.8. Transfer contents of unit Fermenter 1 to Fermenter 2. Transfer 100% of vessel contents. The transfer time is 0.5 day.

1.9. Transfer contents of unit Holding Tank to Fermenter 2 through heat exchanger Heat Exchanger. The final stream temperature is 37 C. Transfer 293.5 kg of vessel contents. The transfer time is 0.5 day.

1.10. Ferment in unit Fermenter 2. The yield of tPA-CHO Cells in the Solid phase is 0.117, of Endotoxin in the Liquid phase is 7.67e-6, of tPA in the Liquid phase is 0.00039, of WATER in the Liquid phase is 0.883, of Media in the Solid phase is 0, of Media in the Liquid phase is 0 and of tPA-CHO Cells in the Liquid phase is 0. The fermentation time is 7 day. Continuously add 0.4 kg of CARBON-DIOXIDE. Continuously add 4.5 kg of AIR.

Series

1.11. Clean unit Fermenter 1. Clean with 60 kg of WATER. The feed time is 1 min. Cleaning time is 20 h.

1.12. Sterilize the contents of Fermenter 1. The sterilization temperature is 130 C. The heat-up time is 1 h. Maintain the temperature for 2 h. The cool-down time is 1 h.

End Parallel

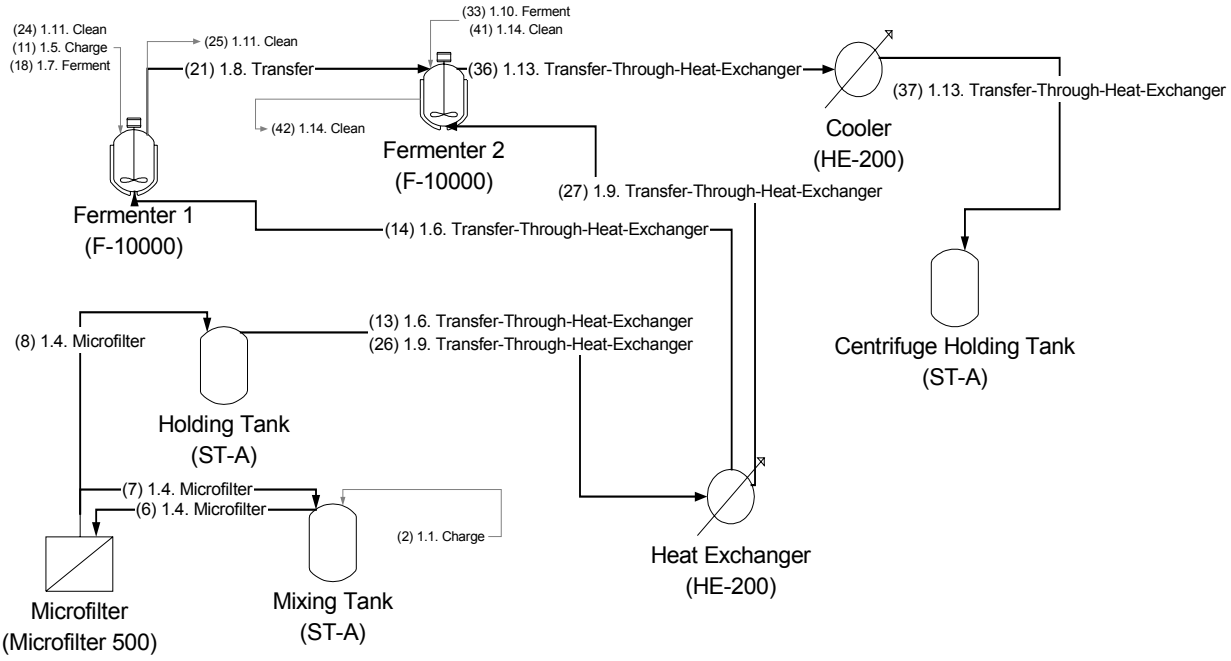
1.13. Transfer contents of unit Fermenter 2 to Centrifuge Holding Tank through heat exchanger Cooler. The final stream temperature is 4 C. Transfer 100% of vessel contents. The transfer time is 0.5 day.

1.14. Clean unit Fermenter 2. Clean with 600 kg of WATER. The feed time is 1 min. Cleaning time is 20 h.

1.15. Sterilize the contents of Fermenter 2. The sterilization temperature is 130 C. The heat-up time is 1 h. Maintain the temperature for 2 h. The cool-down time is 1 h.

a. Operations recipe for the process

Figure 3. BATCH PLUS simulation for Example 1



b. BATCH PLUS simulation flowsheet

Figure 3. BATCH PLUS simulation for Example 1 (Cont'd.)

Step Stream Table

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 12/16/01 17:10

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 0.12 kg

BATCH PLUS Stream Label			1.1. Charge-50	1.1. Charge-51	1.4. Microfilter-55	1.4. Microfilter-56	1.4. Microfilter-57	1.5. Charge-60
Operation			1.1. Charge	1.1. Charge	1.4. Microfilter	1.4. Microfilter	1.4. Microfilter	1.5. Charge
Start Time		(min)	0.00	0.00	4,380.00	4,380.00	4,380.00	4,500.00
End Time		(min)	60.00	60.00	4,500.00	4,500.00	4,500.00	4,560.00
Total Time		(min)	60.00	60.00	120.00	120.00	120.00	60.00
From Unit					Mixing Tank	Microfilter	Microfilter	
To Unit			Mixing Tank	Mixing Tank	Microfilter	Mixing Tank	Holding Tank	Fermenter 1
Stream Type			Input	Input	Intermediate	Intermediate	Intermediate	Input
Mass - (kg)	Per Batch	Mol Wt						
Total			458.3000	3,565.0000	4,023.3000	4,023.3000	4,023.3000	1.2000
Endotoxin		18.02						
tPA		18.02						
AIR		28.95						
CARBON-DIOXIDE		44.01						
WATER		18.02		3,565.0000	3,565.0000	3,565.0000	3,565.0000	
Media		18.02	458.3000		458.3000	458.3000	458.3000	
tPA-CHO Cells		18.02						1.2000
Total Mass		(kg)	458.30	3,565.00	4,023.30	4,023.30	4,023.30	1.20
Total Volume		(liter)	461.15	3,587.19	3,968.36	3,968.36	3,968.36	1.21
Mass Flowrate		(kg/min)	7.64	59.42	33.53	33.53	33.53	0.02
Volume Flowrate		(liter/h)	461.15	3,587.19	1,984.18	1,984.18	1,984.18	1.21
Composite Product Factor			3,721.42	28,947.96	32,669.38	32,669.38	32,669.38	9.74
Phase			Liquid1	Liquid1	Liquid1	Liquid1	Liquid1	Liquid1
Temperature		(C)	25.00	25.00	4.00	4.00	4.00	25.00
Average Density		(kg/Cubic m)	993.81	993.81	1,013.84	1,013.84	1,013.84	993.81
Average Viscosity		(cp)	0.92	0.92	1.53	1.53	1.53	0.92
Average Heat Capacity		(kJ/kg-K)	4.18	4.18	4.21	4.21	4.21	4.18
Average Molecular Weight			18.02	18.02	18.02	18.02	18.02	18.02

c. Stream variables

Figure 3. BATCH PLUS simulation for Example 1 (Cont'd.)

Step Stream Table

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 12/16/2001 17:10

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 0.12 kg

BATCH PLUS Stream Label			1.6. Transfer-Through-Heat-Exchanger-62	1.6. Transfer-Through-Heat-Exchanger-63	1.7. Ferment-67	1.7. Ferment-68	1.8. Transfer-70	1.11. Clean-94
Operation			1.6. Transfer-Through-Heat-Exchanger	1.6. Transfer-Through-Heat-Exchanger	1.7. Ferment	1.7. Ferment	1.8. Transfer	1.11. Clean
Start Time		(min)	4,560.00	4,560.00	5,280.00	5,280.00	12,480.00	13,200.00
End Time		(min)	5,280.00	5,280.00	5,280.30	5,286.76	13,200.00	13,201.00
Total Time		(min)	720.00	720.00	0.30	6.76	720.00	1.00
From Unit			Holding Tank	Heat Exchanger			Fermenter 1	
To Unit			Heat Exchanger	Fermenter 1	Fermenter 1	Fermenter 1	Fermenter 2	Fermenter 1
Stream Type			Intermediate	Intermediate	Input	Input	Intermediate	Input
Mass - (kg)	**Per Batch**	**Mol Wt**						
Total			21.2000	21.2000	0.0200	0.3000	22.4000	60.0000
Endotoxin		18.02					0.0022	
tPA		18.02					0.0022	
AIR		28.95				0.3000		
CARBON-DIOXIDE		44.01			0.0200			
WATER		18.02	18.7851	18.7851			18.9690	60.0000
Media		18.02	2.4149	2.4149				
tPA-CHO Cells		18.02					3.4265	
Total Mass		(kg)	21.20	21.20	0.02	0.30	22.40	60.00
Total Volume		(liter)	20.91	21.58	11.12	253.53	22.73	60.37
Mass Flowrate		(kg/min)	0.03	0.03	0.07	0.04	0.03	37.27
Volume Flowrate		(liter/h)	1.74	1.80	2,250.00	2,250.00	1.89	2,250.00
Composite Product Factor			172.14	172.14	0.16	2.44	181.89	487.20
Phase			Liquid1	Liquid1	Gas	Gas	Liquid1+Solid	Liquid1
Temperature		(C)	4.01	37.00	25.00	25.00	36.31	25.00
Average Density		(kg/Cubic m)	1,013.84	982.17	1.80	1.18	985.41	993.81
Average Viscosity		(cp)	1.53	0.71	0.01	0.02	0.61	0.92
Average Heat Capacity		(kJ/kg-K)	4.21	4.17	0.85	1.00	4.17	4.18
Average Molecular Weight			18.02	18.02	44.01	28.95	18.02	18.02

c. Stream variables (Cont'd.)

Figure 3. BATCH PLUS simulation for Example 1 (Cont'd.)

Step Stream Table

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 12/16/2001 17:10

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 0.12 kg

BATCH PLUS Stream Label			1.11. Clean-95	1.9. Transfer-Through-Heat-Exchanger-96	1.9. Transfer-Through-Heat-Exchanger-97	1.10. Ferment-103	1.10. Ferment-104	1.13. Transfer-Through-Heat-Exchanger-106
Operation			1.11. Clean	1.9. Transfer-Through-Heat-Exchanger	1.9. Transfer-Through-Heat-Exchanger	1.10. Ferment	1.10. Ferment	1.13. Transfer-Through-Heat-Exchanger
Start Time		(min)	14,401.00	13,200.00	13,200.00	13,920.00	13,920.00	24,000.00
End Time		(min)	14,402.00	13,920.00	13,920.00	13,925.93	14,021.41	24,720.00
Total Time		(min)	1.00	720.00	720.00	5.93	101.41	720.00
From Unit			Fermenter 1	Holding Tank	Heat Exchanger			Fermenter 2
To Unit				Heat Exchanger	Fermenter 2	Fermenter 2	Fermenter 2	Cooler
Stream Type			Output	Intermediate	Intermediate	Input	Input	Intermediate
Mass - (kg)	**Per Batch**	**Mol Wt**						
Total			60.0000	293.5000	293.5000	0.4000	4.5000	315.9000
Endotoxin		18.02						0.0024
tPA		18.02						0.1232
AIR		28.95					4.5000	
CARBON-DIOXIDE		44.01				0.4000		
WATER		18.02	60.0000	260.0670	260.0670			278.8288
Media		18.02		33.4330	33.4330			
tPA-CHO Cells		18.02						36.9456
Total Mass		(kg)	60.00	293.50	293.50	0.40	4.50	315.90
Total Volume		(liter)	60.37	289.49	298.83	222.36	3,802.88	320.94
Mass Flowrate		(kg/min)	37.27	0.41	0.41	0.07	0.04	0.44
Volume Flowrate		(liter/h)	2,250.00	24.12	24.90	2,250.00	2,250.00	26.75
Composite Product Factor			487.20	2,383.23	2,383.23	3.25	36.54	2,565.12
Phase			Liquid1	Liquid1	Liquid1	Gas	Gas	Liquid1+Solid
Temperature		(C)	25.00	4.01	37.00	25.00	25.00	36.90
Average Density		(kg/Cubic m)	993.81	1,013.84	982.17	1.80	1.18	984.29
Average Viscosity		(cp)	0.92	1.53	0.71	0.01	0.02	0.63
Average Heat Capacity		(kJ/kg-K)	4.18	4.21	4.17	0.85	1.00	4.17
Average Molecular Weight			18.02	18.02	18.02	44.01	28.95	18.02

c. Stream variables (Cont'd.)

Figure 3. BATCH PLUS simulation for Example 1 (Cont'd.)

Step Stream Table

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 12/16/2001 17:10

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 0.12 kg

BATCH PLUS Stream Label			1.13. Transfer-Through-Heat-Exchanger-107	1.14. Clean-111	1.14. Clean-112
Operation			1.13. Transfer-Through-Heat-Exchanger	1.14. Clean	1.14. Clean
Start Time		(min)	24,000.00	24,720.00	25,921.00
End Time		(min)	24,720.00	24,721.00	25,922.00
Total Time		(min)	720.00	1.00	1.00
From Unit			Cooler		Fermenter 2
To Unit			Centrifuge Holding Tank	Fermenter 2	
Stream Type			Intermediate	Input	Output
Mass - (kg)	Per Batch	Mol Wt			
Total			315.9000	600.0000	600.0000
Endotoxin		18.02	0.0024		
tPA		18.02	0.1232		
AIR		28.95			
CARBON-DIOXIDE		44.01			
WATER		18.02	278.8288	600.0000	600.0000
Media		18.02			
tPA-CHO Cells		18.02	36.9456		
Total Mass		(kg)	315.90	600.00	600.00
Total Volume		(liter)	312.09	603.73	603.73
Mass Flowrate		(kg/min)	0.44	37.27	37.27
Volume Flowrate		(liter/h)	26.01	2,250.00	2,250.00
Composite Product Factor			2,565.12	4,872.03	4,872.03
Phase			Liquid1+Solid	Liquid1	Liquid1
Temperature		(C)	4.00	25.00	25.00
Average Density		(kg/Cubic m)	1,012.20	993.81	993.81
Average Viscosity		(cp)	1.35	0.92	0.92
Average Heat Capacity		(kJ/kg-K)	4.21	4.18	4.18
Average Molecular Weight			18.02	18.02	18.02

c. Stream variables (Cont'd.)

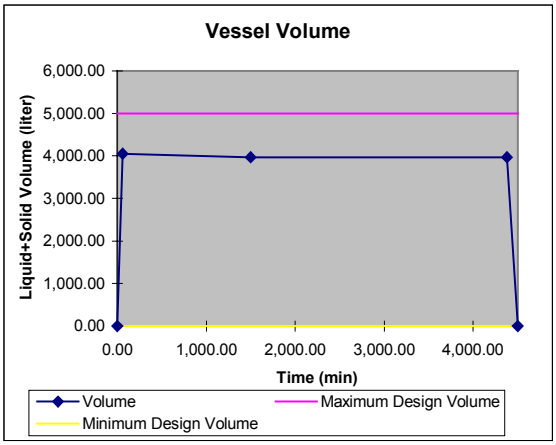
Figure 3. BATCH PLUS simulation for Example 1 (Cont'd.)

Step Equipment Contents

Process (Version):	Reactor Growth Chain (1.0)	**Key Input Intermediate:**	Media	
Step (Version):	Step1 (1.0)	**Key Output Intermediate:**	tPA	
Simulation Date:	12/16/01 17:10	**Number of Batches:**	1	
		Plan Quantity:	0.12	kg

Mixing Tank

Operation		START	1.1. Charge	1.2. Cool	1.3. Age	1.4. Microfilter
Time	**(min)**	0.00	60.00	1,500.00	4,380.00	4,500.00
Mass - (kg)	**Mol Wt**					
Total		5.8957	4,024.4221	4,024.6086	4,024.6086	6.3424
WATER	18.02		3,565.0000	3,565.0000	3,565.0000	
NITROGEN	28.01	4.5223	0.8607	1.0038	1.0038	4.8650
OXYGEN	32.00	1.3734	0.2614	0.3048	0.3048	1.4774
Media	18.02		458.3000	458.3000	458.3000	
Liquid+Solid Mass	**(kg)**	0.00	4,023.30	4,023.30	4,023.30	0.00
Liquid+Solid Volume	**(liter)**	0.00	4,048.34	3,968.36	3,968.36	0.00
Phase		Gas	Gas+Liquid1	Gas+Liquid1	Gas+Liquid1	Gas
Temperature	**(C)**	25.00	25.00	4.00	4.00	4.00
Pressure	**(kPa)**	101.33	101.33	101.33	101.33	101.33
Average Liq+Sol Density	**(kg/Cubic m)**	0.00	993.81	1,013.84	1,013.84	0.00
Average Liq+Sol Viscosity	**(cp)**	0.00	0.92	1.53	1.53	0.00
Average Liq+Sol Heat Capacity	**(kJ/kg-K)**	0.00	4.18	4.21	4.21	0.00
Average Liq+Sol Molecular Weight		0.00	18.02	18.02	18.02	0.00



d. Mixing Tank report

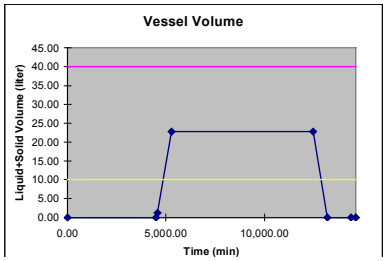
Figure 3. BATCH PLUS simulation for Example 1 (Cont'd.)

Step Equipment Contents

Process (Version):	Reactor Growth Chain (1.0)	**Key Input Intermediate:**	Media	
Step (Version):	Step1 (1.0)	**Key Output Intermediate:**	tPA	
Simulation Date:	1/14/2002 17:40	**Number of Batches:**	1	
		Plan Quantity:	0.12	kg

Fermenter 1

Operation		START	1.5. Charge	1.5. Charge	1.6. Transfer-Through-Heat-Exchanger	1.7. Ferment	1.8. Transfer	1.11. Clean
Time	**(min)**	0.00	4,500.00	4,560.00	5,280.00	12,480.00	13,200.00	14,402.00
Mass - (kg)	**Mol Wt**							
Total		0.0472	0.0472	1.2457	22.4195	22.4201	0.0459	0.0459
Endotoxin	18.02					0.0022		
tPA	18.02					0.0022		
AIR	28.95					0.0177	0.0177	0.0177
CARBON-DIOXIDE	44.01					0.0012	0.0012	0.0012
WATER	18.02				18.7851	18.9690		
NITROGEN	28.01	0.0362	0.0362	0.0351	0.0150	0.0009	0.0207	0.0207
OXYGEN	32.00	0.0110	0.0110	0.0107	0.0046	0.0003	0.0063	0.0063
Media	18.02				2.4149			
tPA-CHO Cells	18.02			1.2000	1.2000	3.4265		
Liquid+Solid Mass	**(kg)**	0.00	0.00	1.20	22.40	22.40	0.00	0.00
Liquid+Solid Volume	**(liter)**	0.00	0.00	1.21	22.79	22.73	0.00	0.00
Phase		Gas	Gas	Gas+Liquid1	Gas+Liquid1	Gas+Liquid1+Solid	Gas	Gas
Temperature	**(C)**	25.00	25.00	25.00	36.35	36.31	36.31	36.31
Pressure	**(kPa)**	101.33	101.33	101.33	101.33	101.33	101.32	101.32
Average Liq+Sol Density	**(kg/Cubic m)**	0.00	0.00	993.81	982.80	985.41	0.00	0.00
Average Liq+Sol Viscosity	**(cp)**	0.00	0.00	0.92	0.72	0.72	0.00	0.00
Average Liq+Sol Heat Capacity	**(kJ/kg-K)**	0.00	0.00	4.18	4.17	4.17	0.00	0.00
Average Liq+Sol Molecular Weight		0.00	0.00	18.02	18.02	18.02	0.00	0.00



e. Fermenter 1 report

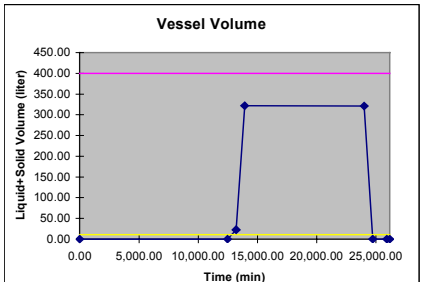
Figure 3. BATCH PLUS simulation for Example 1 (Cont'd.)

Step Equipment Contents

Process (Version):	Reactor Growth Chain (1.0)	**Key Input Intermediate:**	Media	
Step (Version):	Step1 (1.0)	**Key Output Intermediate:**	tPA	
Simulation Date:	1/14/2002 17:40	**Number of Batches:**	1	
		Plan Quantity:	0.12	kg

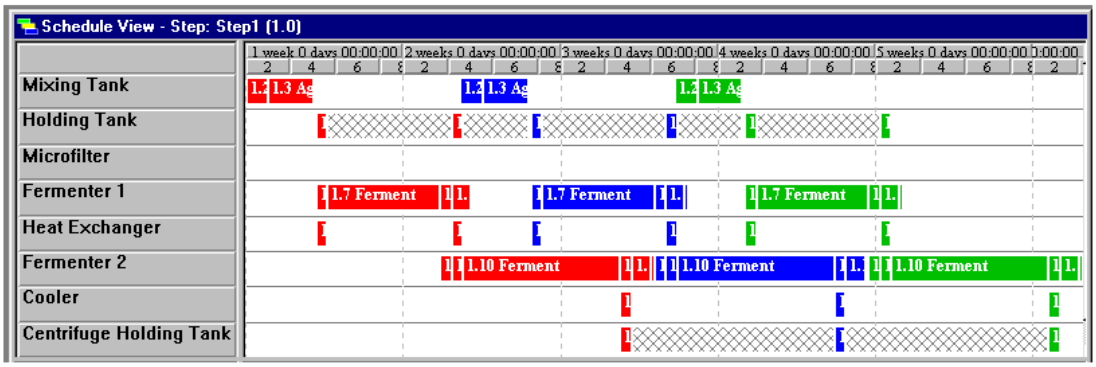
Fermenter 2

Operation		START	1.8. Transfer	1.8. Transfer	1.9. Transfer-Through-Heat-Exchanger	1.10. Ferment	1.13. Transfer-Through-Heat-Exchanger	1.14. Clean
Time	**(min)**	0.00	12,480.00	13,200.00	13,920.00	24,000.00	24,720.00	25,922.00
Mass - (kg)	**Mol Wt**							
Total		0.4717	0.4717	22.8287	315.9889	315.9925	0.4564	0.4564
Endotoxin	18.02			0.0022	0.0022	0.0024		
tPA	18.02			0.0022	0.0022	0.1232		
AIR	28.95					0.0834	0.0834	0.0834
CARBON-DIOXIDE	44.01					0.0074	0.0074	0.0074
WATER	18.02			18.9690	279.0360	278.8288		
NITROGEN	28.01	0.3618	0.3618	0.3288	0.0682	0.0013	0.2804	0.2804
OXYGEN	32.00	0.1099	0.1099	0.0999	0.0207	0.0004	0.0852	0.0852
Media	18.02				33.4330			
tPA-CHO Cells	18.02			3.4265	3.4265	36.9456		
Liquid+Solid Mass	**(kg)**	0.00	0.00	22.40	315.90	315.90	0.00	0.00
Liquid+Solid Volume	**(liter)**	0.00	0.00	22.73	321.56	320.94	0.00	0.00
Phase		Gas	Gas	Gas+Liquid1+Solid	Gas+Liquid1+Solid	Gas+Liquid1+Solid	Gas	Gas
Temperature	**(C)**	25.00	25.00	36.26	36.95	36.90	36.90	36.90
Pressure	**(kPa)**	101.33	101.33	101.33	101.33	101.33	101.33	101.33
Average Liq+Sol Density	**(kg/Cubic m)**	0.00	0.00	985.46	982.41	984.29	0.00	0.00
Average Liq+Sol Viscosity	**(cp)**	0.00	0.00	0.72	0.71	0.72	0.00	0.00
Average Liq+Sol Heat Capacity	**(kJ/kg-K)**	0.00	0.00	4.17	4.17	4.17	0.00	0.00
Average Liq+Sol Molecular Weight		0.00	0.00	18.02	18.02	18.02	0.00	0.00



f. Fermenter 2 report

Figure 3. BATCH PLUS simulation for Example 1 (Cont'd.)



g. 3-batch Gantt chart

Figure 3. BATCH PLUS simulation for Example 1 (Cont'd.)

EXER. 1 Debottlenecking Reactor Train. When the third tPA cultivator in Section 2.4 is added to the two cultivators in Example 1, as shown in Figure 1a, a significant time strain is placed on the process because the combined feed, cultivation, harvest, and cleaning time in this largest vessel is long and rigid. Consequently, the remainder of the process is designed to keep this cultivator in constant use, so as to maximize the yearly output of product. Note that, in many cases, when an equipment item causes a bottleneck, a duplicate is installed so as to reduce the cycle time.

For this exercise, the third cultivator is added to the simulation in Example 1, with the specifications for the mixer, filter, holding tank, heat exchanger 1, and first two cultivators identical to those in Example 1. After the cultivation is completed in Cultivator 2, its cell mass is transferred as inoculum to Cultivator 3 over 0.5 day. Then, the remaining media from the mixing tank is heated to 37°F and added over 1.5 day, after which cultivation takes place over eight days. Immediately after the transfer from Cultivator 2 to Cultivator 3, Cultivator 2 is cleaned-in-place using 600 Kg of water over 20 hours. The yield of the cultivation in Cultivator 3 is 11.4 wt% tPA-CHO cells, 7.7×10^{-5} wt% endotoxin, 88.9 wt% water, and 0.0559 wt% tPA. When the cultivation is completed in Cultivator 3, its contents are cooled in a heat exchanger to 4°C and transferred to the centrifuge holding tank over one day, and Cultivator 3 is cleaned using 600 Kg of water over 67 hours and sterilized using the procedure for Cultivators 1 and 2.

To eliminate an undesirable bottleneck(s), and reduce the cycle time to 14 days (total operation time of Fermenter 3), it may be necessary to add an equipment unit(s).

Print and submit the text recipes and 3-batch schedules for both the original process and the modified process, if debottlenecking is necessary, as prepared by BATCH PLUS.

EXER. 2 tPA Process Simulation. For the entire process flowsheet in Figure 7a,b,c of Section 2.4, complete a BATCH PLUS simulation. Print and submit the text recipes and 3-batch schedules for both the original process and the modified process, as prepared by BATCH PLUS.

Note that the operating times, batch sizes, and recovery percentages are shown in Figure 7a,b,c. Unfortunately, BATCH PLUS determines that 15,000 Kg/batch of elution buffer are required to elute the affinity chromatography column. To circumvent this, after 523 Kg have been fed, specify a "cut" of 404 Kg. The latter is collected as the elution effluent while the difference is rejected in the wastewater. Also, BATCH PLUS does not model the selective adsorption of endotoxin without tPA, arginine, sucrose, glycine, NaCl, and NaOH. Consequently, to obtain the desired effluent streams, one approach is to adsorb endotoxin, while recovering the waste effluent (which contains tPA, arginine, sucrose, glycine, NaCl, and NaOH). Then, the elution buffer (500 Kg/batch of water) is used to elute the endotoxin, which is rejected as wastewater.

BATCH PLUS INSTRUCTIONS FOR EXAMPLE 1

To assist new users, the following step-by-step instructions are provided for using BATCH PLUS to solve Example 1. Before attempting Exercises 1 and 2, it may be helpful to obtain the solution to Example 1 using these instructions.

1) Open BATCH PLUS.
2) Highlight *Create a New Blank Project* and then click *OK*.
3) Enter **Example Problem** in the *Project Name* area. Click *OK*.
4) Click *Data* on the *main menu* toolbar and choose *Network Select/Materials…*

   The list on the right displays the materials (or chemical species) that have been selected for the process. Nitrogen, oxygen and water are the defaults. It will be necessary to define additional materials for our process.

   Click *Cancel*.
5) From the *Data* menu, select *Pure Components*…
6) The *Pure Component* profile for oxygen is now displayed. From the *component* drop-down menu, select the profile for water. Note that for the tPA process, the major components are modeled using the physical properties of water.
7) With the profile for water being displayed, click the *New Same As* button. Enter **tPA-CHO Cells** as the new component name. Click *Update*. Repeat this process to create a new component named **Media**, and again to create components named **tPA** and **Endotoxin**. Click *OK* to close the box.

8) From the *Data* menu, choose *Network Select/Materials*… Scroll down the list of *Source Materials* on the left, and choose **Carbon Dioxide** and **Air** to add to the list of components for our process. Click *OK*.

9) Click the *New Process* icon. Enter **tPA Production** as the process name. The key raw material should be selected as **Media**, and the final product as **tPA**. Click *OK*.

10) Click the *New Step* button. The default *step name* is **Step1**. Select **Media** as the *key input intermediate*, and **tPA** as the *key output intermediate*. Click *OK*.

11) Click the *Text Recipe* button. The *Add a Unit Procedure* dialog box appears. Name the unit procedure **Reactor Growth Chain**. For the *primary equipment unit*, select **Fermenter F-10000**. Click the *"..."* button next to the selection. The *Equipment* dialog box appears. Press the *New Same As* button and enter the *Equipment/Stage Name* **Fermenter 1**. Note that this creates an image of the **Fermenter F-10000** unit, with the name **Fermenter 1**. Click the *Detail* tab to change the **capacity** to **40 liters**. Click *Update*. Press *New Same As* again and enter the *Equipment/Stage Name* **Fermenter 2**. Change the **capacity** of this unit to **400 liters**. Click Update, then *OK*. **Fermenter 2** should be the *primary equipment unit*. Click *OK*.

12) In the *Recipe Text* window, right click and select Insert *Batch Operation (A-L) / **Charge***… Double click on "Charge…" The *Charge Operation* dialog box appears. Select **ST-A** as the unit to be charged, and press the "..." button. Press *New Same As* and to create its image with the name **Mixing Tank**. Now click *Update*, then *OK*. In line 1, specify that **458.3 kg** of **media** is to be charged. Press the *Detail* button on line 1. Enter a charge time of **1 h** and press *OK*. On line 2, specify that **3565 kg** of

water is to be charged. Use the *Detail* button on line 2 to specify a charge time of **1 h** for the water. Click *OK*.

13) Insert the *batch operation* **Cool**. Cool the unit **Mixing Tank** to **4C**. Click the *Optional* tab and set the cooling time to **1 day**.

14) Insert the next *batch operation* **Age**. Age the contents of **Mixing Tank** for **2 days**.

15) Insert the *batch operation* **Microfilter**. Microfilter the contents of **Mixing Tank** in **Microfilter 500**. Define an image of the latter unit with the name, **Microfilter**, by pressing the *New Same As* button and entering **Microfilter.** The *operation mode* should be batch concentration. Press the *Separation* button.

    For this model, the trace quantities of bacteria are not represented as a component. Since the model does not account for the removal of bacteria, no component separations are specified.

    Choose the selection that places all unspecified components in the **Permeate**. Click *OK*, and then click the *Optional* tab. Specify the operating time as **2 h**. Click the *Permeate Stream* button. The permeate stream should be sent to an image of the unit **ST-B,** which is named **Holding Tank** using the *New Same As* button. Click *OK* to close the dialog boxes.

16) Insert the *batch operation* **Charge**, and charge **Fermenter 1** with **1.2 kg** of **tPA-CHO Cells**. Enter a charge time of **1 h**.

17) Insert the *batch operation* **Transfer-Through-Heat-Exchanger**. Transfer the contents of **Holding Tank** to **Fermenter 1** through an image of **HE-200** named **Heat Exchanger** (using the *New Same As* button). The final stream temperature is

37°C. Press the *Optional* tab, and then *Transfer Stream...* to set the transfer time as **0.5 day** and the transfer amount as **21.2 kg**.

18) Select the *batch operation* **Ferment**. In the *Ferment Operation* dialog box, select **Fermenter 1** as the fermentation unit. Specify the yield of **tPA-CHO Cells** in the **liquid** phase as **0**, the yield of **tPA-CHO Cells** in the **solid** phase as **0.153**, the yield of **water** in the **liquid** phase as **0.847**, the yield of **tPA** in the **liquid** phase as **0.0001**, and the yield of **Endotoxin** in the **liquid** phase as **.0001**. The yield of **Media** in the **liquid** and **solid** phases are both **zero**. Select the *Optional* tab. Specify a fermentation time of **5 day**. Press the *Feed* button. On line 1, enter that **0.02 kg of carbon dioxide** are to be fed. On line 2, enter that **0.3 kg of air** are to be added. Press *OK* to close the dialog boxes.

19) **Fermenter 2** begins its fermentation procedure as the cleaning of **Fermenter 1** is initiated. Special steps are required to allow for these simultaneous actions. Right click on the *Recipe Text* entry 1.7 and select *Insert Parallel*. Highlight the first word **Series** and then right click to insert the **Transfer** operation. Transfer **100%** of the contents of **Fermenter 1** to **Fermenter 2**. The transfer time is **0.5 day**.

20) Insert *batch operation* **Transfer-Through-Heat-Exchanger** and specify that **293.5 kg** of the contents of the **Holding Tank** are transferred to **Fermenter 2** through the **Heat Exchanger** with an outlet temperature of **37°C**, and a transfer time of **0.5 day**.

21) Insert *batch operation* **Ferment**. In the *Ferment Operation* dialog box, **Fermenter 2** should be selected as the fermentation vessel. The yield of **tPA-CHO Cells** in the **solid** phase is **0.117**, the yield of **endotoxin** in the **liquid** phase is **7.67e-6**, the yield of **tPA** in the **liquid** phase is **0.00039**, the yield of **water** in the **liquid** phase is **0.883**,

the yield of **tPA-CHO Cells** in the **liquid** phase is **0**, and the yield of **media** in the **liquid** and **solid** phases are both **0**. Click on the *Optional* tab. The fermentation time is **7 days**. Press the *Feed* button. In lines 1 and 2, indicate that **4.5 kg of air** and **0.4 kg of carbon dioxide** are to be fed.

22) Highlight the second **Series** keyword and right click to insert the *batch operation* **Clean**. Unit **Fermenter 1** is to be cleaned. Click the *Optional* tab to specify a cleaning time of **20 hours** and a cleaning medium of **60 kg** of **water**. Click the *Details…* button to specify a charge time of **1 min**.

23) Add the procedure **sterilize**, to sterilize the contents of **Fermenter 1** at **130ºC**. Click the *Optional* tab to select **heat-up, maintain,** and **cool-down** times of **1**, **2**, and **1 hours** respectively.

24) Highlight the keyword **End Parallel** and double click to insert the procedure **Transfer-Through-Heat-Exchanger**. The contents of **Fermenter 2** are to be transferred to a storage tank named **Centrifuge Holding Tank** through a heat exchanger named **Cooler**. The final stream temperature is **4ºC**. Click the *Optional* tab and then the *Transfer Stream* button. Enter a transfer time of **0.5 day,** and a transfer amount of **100 percent**.

25) Add the *procedure* **Clean**. Clean the contents of **Fermenter 2** for **20 hours** with **600 kg** of **water**, and a **1 min**. charge time.

26) Finally, **sterilize** the contents of **Fermenter 2** in the same manner as **Fermenter 1**.

27) The *Recipe Text* is now complete. In the main toolbar, click the *Save Project* icon to save the recipe.

28) In the main menu, select *Run / Simulate Batch*. Select *The Entire Recipe* to be simulated and press *OK*.

29) In the *Results* menu, select *Schedule* to display a single-batch Gantt chart.

30) When the chart is displayed, locate the *Extrapolate Multiple Batches* icon in the toolbar next to the magnifying glass icons. Depress this icon.

    Notice that the dialog box indicates a minimum batch time of 9 days, 12 hours and 2 minutes. This is the batch time of Fermenter 2 (8 days of fermentation, 0.5 day for transferring the product, 20 hours and 2 minutes for cleaning and 4 hours for sterilization).

    Select the *Number of Batches* to be extrapolated as **3**. Press *OK*.

    It should be evident by looking at the chart that the bottleneck in the process is Fermenter 2. The other vessels are idle for periods of time, while Fermenter 2 is in use at all times.

    Close the *Schedule View* window.

31) Under the *Results* menu, choose *Excel Reports / Equipment Contents*.

    Use the tabs on the bottom of the EXCEL spreadsheet to examine the contents of the different vessels. This report contains information to determine the required vessel sizes. The maximum liquid and solid volume of the **Mixing Tank** is 4,050 L, with the maximum for **Fermenter 2**, 322 L. On this basis, prudent vessel sizes would be 5,000 and 400 L, respectively.

Solution to Exercise 1, Section 3.5

Two solutions are presented, the first with a bottleneck, the second with the bottleneck removed.

Bottleneck

In this simulation, just one holding tank and heat exchanger are used to feed the three cultivators, which are fed in sequence, one after the other. After the holding tank is charged, and the contents aged, Fermenter 1 is fed. Then, the holding tank remains idle for several days before the feeding of Fermenter 2 begins. Again, the holding tank sits idle for several more days before the feeding of Fermenter 3 begins. As shown in the results (from which the *recipe text* and *Gantt chart* are shown on the next pages), the large occupancy time of this vessel limits the batch time to 15 days, 15 hours. This means that the largest bioreactor, Fermenter 3, sits idle over 1.5 day per batch, an inefficient use of process equipment.

Bottleneck Solution - Recipe

1. Reactor Growth Chain

1.1. Charge Mixing Tank with 458.3 kg of Media. The charge time is 1 h. Charge Mixing Tank with 3565 kg of WATER. The charge time is 1 h.

1.2. Cool unit Mixing Tank to 4 C. The cooling time is 1 day.

1.3. Age the contents of unit Mixing Tank for 2 day.

1.4. Microfilter the contents of Mixing Tank in Microfilter. The mode of operation is Batch Concentration. Unspecified components go to the Permeate. The operation time is 2 h. The permeate stream is sent to Holding Tank.

1.5. Charge Fermenter 1 with 1.2 kg of tPA-CHO Cells. The charge time is 1 h.

1.6. Transfer contents of unit Holding Tank to Fermenter 1 through heat exchanger Heat Exchanger. The final stream temperature is 37 C. Transfer 21.2 kg of vessel contents. The transfer time is 0.5 day.

1.7. Ferment in unit Fermenter 1. The yield of tPA-CHO Cells in the Solid phase is 0.153, of Endotoxin in the Liquid phase is 0.0001, of tPA in the Liquid phase is 0.0001, of WATER in the Liquid phase is 0.847, of Media in the Liquid phase is 0, of Media in the Solid phase is 0 and of tPA-CHO Cells in the Liquid phase is 0. The fermentation time is 5 day. Continuously add 0.02 kg of CARBON-DIOXIDE. Continuously add 0.3 kg of AIR.

Start Parallel

Series

1.8. Transfer contents of unit Fermenter 1 to Fermenter 2. Transfer 100% of vessel contents. The transfer time is 0.5 day.

1.9. Transfer contents of unit Holding Tank to Fermenter 2 through heat exchanger Heat Exchanger. The final stream temperature is 37 C. Transfer 293.5 kg of vessel contents. The transfer time is 0.5 day.

1.10. Ferment in unit Fermenter 2. The yield of tPA-CHO Cells in the Solid phase is 0.117, of Endotoxin in the Liquid phase is 7.67e-6, of tPA in the Liquid phase is 0.00039, of WATER in the Liquid phase is 0.883, of Media in the Solid phase is 0, of Media in the Liquid phase is 0 and of tPA-CHO Cells in the Liquid phase is 0. The fermentation time is 7 day. Continuously add 0.4 kg of CARBON-DIOXIDE. Continuously add 4.5 kg of AIR.

Series

1.11. Clean unit Fermenter 1. Clean with 60 kg of WATER. The feed time is 1 min. Cleaning time is 20 h.

1.12. Sterilize the contents of Fermenter 1. The sterilization temperature is 130 C. The heat-up time is 1 h. Maintain the temperature for 2 h. The cool-down time is 1 h.

End Parallel

2. *Final Fermentation*

Start Parallel

Series

2.1. Transfer contents of unit Fermenter 2 to Fermenter 3. Transfer 100% of vessel contents. The transfer time is 0.5 day.

2.2. Transfer contents of unit Holding Tank to Fermenter 3 through heat exchanger Heat Exchanger. The final stream temperature is 37 C. Transfer 100% of vessel contents. The transfer time is 1.5 day.

2.3. Ferment in unit Fermenter 3. The yield of tPA-CHO Cells in the Solid phase is 0.114, of Endotoxin in the Liquid phase is 7.7e-7, of tPA in the Liquid phase is 0.000559, of WATER in the Liquid phase is 0.889, of Media in the Solid phase is 0, of Media in the Liquid phase is 0 and of tPA-CHO Cells in the Liquid phase is 0. The fermentation time is 8 day. The final temperature is 37 C. Continuously add 70 kg of AIR. Continuously add 5.5 kg of CARBON-DIOXIDE.

Series

2.4. Clean unit Fermenter 2. Clean with 600 kg of WATER. The feed time is 1 min. Cleaning time is 20 h.

2.5. Sterilize the contents of Fermenter 2. The sterilization temperature is 130 C. The heat-up time is 1 h. Maintain the temperature for 2 h. The cool-down time is 1 h.

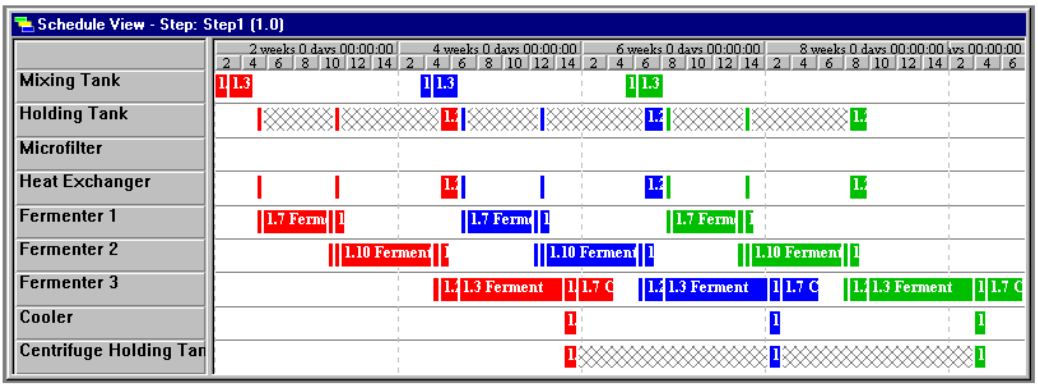
End Parallel

2.6. Transfer contents of unit Fermenter 3 to Centrifuge Holding Tank through heat exchanger Cooler. The final stream temperature is 4 C. Transfer 100% of vessel contents. The transfer time is 1 day.

2.7. Clean unit Fermenter 3. Clean with 600 kg of WATER. The feed time is 1 min. Cleaning time is 68 h.

2.8. Sterilize the contents of Fermenter 3. The sterilization temperature is 130 C. The heat-up time is 1 h. Maintain the temperature for 2 h. The cool-down time is 1 h.

Bottleneck Solution – Gantt Chart



Bottleneck Removed

To increase the yearly production of product, the bottleneck is removed by investing in additional equipment, which is justified, given the high selling price of tPA. This is achieved by adding Holding Tank 2, and its associated heat exchanger, Heat Exchanger 2. The full load of media and water is charged to and aged in Holding Tank 1. After Holding Tank 1 and Heat Exchanger 1 are used to fill Fermenters 1 and 2, the remaining contents are transferred to Holding Tank 2. Fermenter 3 is subsequently fed from this tank, through Heat Exchanger 2. With this transfer, Holding Tank 1 is free substantially earlier, so that it can be charged and the mixture aged earlier for the next batch. With this modification, as shown in the results that follow, the batch time is reduced to 14 days, the uptime of Fermenter 3, a time savings of over 10%.

Note that other arrangements of holding tanks and heat exchangers can achieve a batch time of 14 days.

Solution Without Bottleneck - Recipe

1. Reactor Growth Chain

1.1. Charge Mixing Tank with 458.3 kg of Media. The charge time is 1 h. Charge Mixing Tank with 3565 kg of WATER. The charge time is 1 h.

1.2. Cool unit Mixing Tank to 4 C. The cooling time is 1 day.

1.3. Age the contents of unit Mixing Tank for 2 day.

1.4. Microfilter the contents of Mixing Tank in Microfilter. The mode of operation is Batch Concentration. Unspecified components go to the Permeate. The operation time is 2 h. The permeate stream is sent to Holding Tank 1.

1.5. Charge Fermenter 1 with 1.2 kg of tPA-CHO Cells. The charge time is 1 h.

1.6. Transfer contents of unit Holding Tank 1 to Fermenter 1 through heat exchanger Heat Exchanger 1. The final stream temperature is 37 C. Transfer 21.2 kg of vessel contents. The transfer time is 0.5 day.

1.7. Ferment in unit Fermenter 1. The yield of tPA-CHO Cells in the Solid phase is 0.153, of Endotoxin in the Liquid phase is 0.0001, of tPA in the Liquid phase is 0.0001, of WATER in the Liquid phase is 0.847, of Media in the Liquid phase is 0, of Media in the Solid phase is 0 and of tPA-CHO Cells in the Liquid phase is 0. The fermentation time is 5 day. Continuously add 0.02 kg of CARBON-DIOXIDE. Continuously add 0.3 kg of AIR.

Start Parallel

Series

1.8. Transfer contents of unit Fermenter 1 to Fermenter 2. Transfer 100% of vessel contents. The transfer time is 0.5 day.

1.9. Transfer contents of unit Holding Tank 1 to Fermenter 2 through heat exchanger Heat Exchanger 1. The final stream temperature is 37 C. Transfer 293.5 kg of vessel contents. The transfer time is 0.5 day.

1.10. Ferment in unit Fermenter 2. The yield of tPA-CHO Cells in the Solid phase is 0.117, of Endotoxin in the Liquid phase is 7.67e-6, of tPA in the Liquid phase is 0.00039, of WATER in the Liquid phase is 0.883, of Media in the Solid phase is 0, of Media in the Liquid phase is 0 and of tPA-CHO Cells in the Liquid phase is 0. The fermentation time is 7 day. Continuously add 0.4 kg of CARBON-DIOXIDE. Continuously add 4.5 kg of AIR.

1.11. Transfer contents of unit Holding Tank 1 to Holding Tank 2.

Series

1.12. Clean unit Fermenter 1. Clean with 60 kg of WATER. The feed time is 1 min. Cleaning time is 20 h.

1.13. Sterilize the contents of Fermenter 1. The sterilization temperature is 130 C. The heat-up time is 1 h. Maintain the temperature for 2 h. The cool-down time is 1 h.

End Parallel

2. Final Fermentation

Start Parallel

Series

2.1. Transfer contents of unit Fermenter 2 to Fermenter 3. Transfer 100% of vessel contents. The transfer time is 0.5 day.

2.2. Transfer contents of unit Holding Tank 2 to Fermenter 3 through heat exchanger Heat Exchanger 2. The final stream temperature is 37 C. Transfer 100% of vessel contents. The transfer time is 1.5 day.

2.3. Ferment in unit Fermenter 3. The yield of tPA-CHO Cells in the Solid phase is 0.114, of Endotoxin in the Liquid phase is 7.7e-7, of tPA in the Liquid phase is 0.000559, of WATER in the Liquid phase is 0.889, of Media in the Solid phase is 0, of Media in the Liquid phase is 0 and of tPA-CHO Cells in the Liquid phase is 0. The fermentation time is 8 day. Continuously add 70 kg of AIR. Continuously add 5.5 kg of CARBON-DIOXIDE.

Series

2.4. Clean unit Fermenter 2. Clean with 600 kg of WATER. The feed time is 1 min. Cleaning time is 20 h.

2.5. Sterilize the contents of Fermenter 2. The sterilization temperature is 130 C. The heat-up time is 1 h. Maintain the temperature for 2 h. The cool-down time is 1 h.

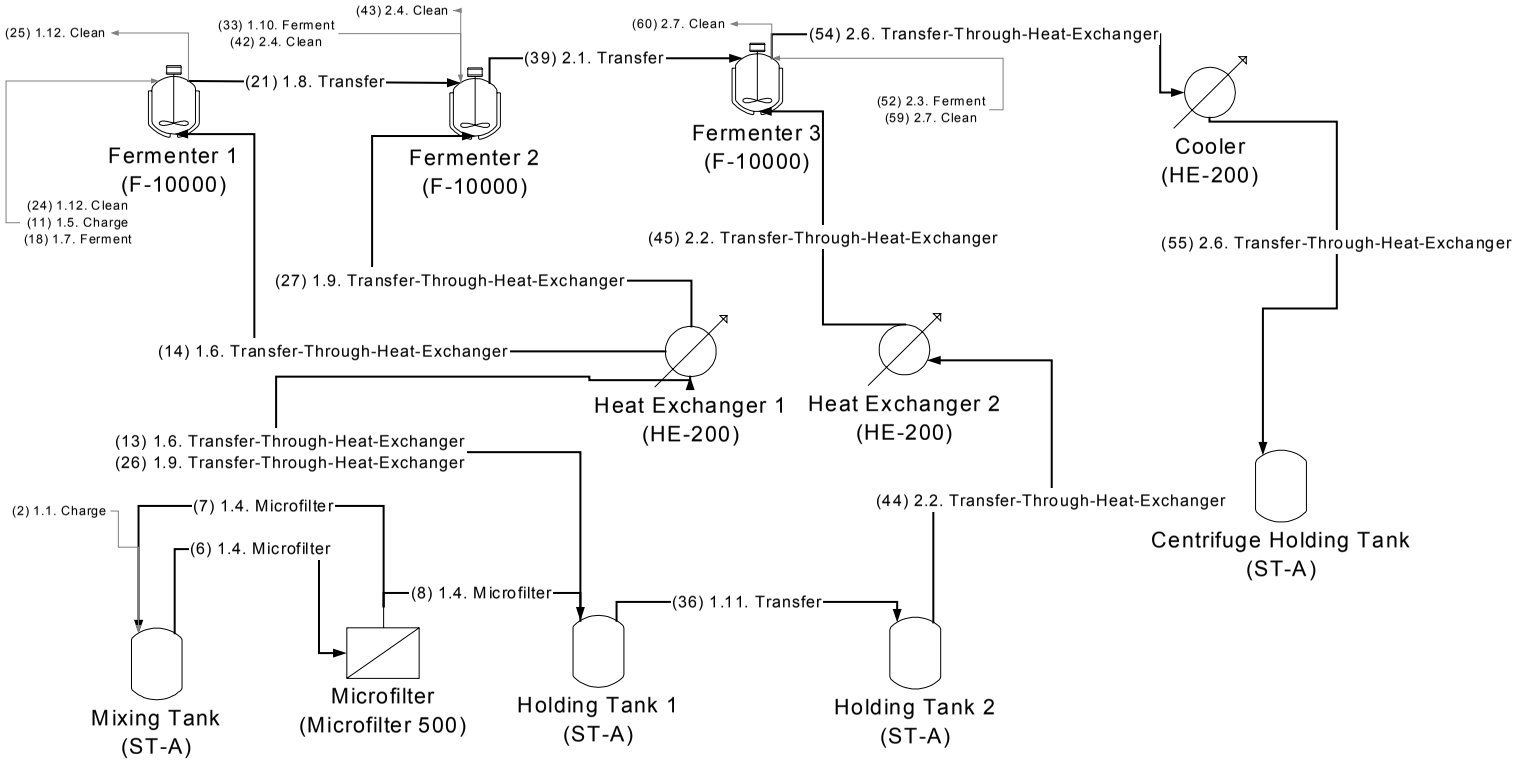
End Parallel

2.6. Transfer contents of unit Fermenter 3 to Centrifuge Holding Tank through heat exchanger Cooler. The final stream temperature is 4 C. The transfer time is 1 day.

2.7. Clean unit Fermenter 3. Clean with 600 kg of WATER. The feed time is 1 min. Cleaning time is 68 h.

2.8. Sterilize the contents of Fermenter 3. The sterilization temperature is 130 C. The heat-up time is 1 h. Maintain the temperature for 2 h. The cool-down time is 1 h.

Solution Without Bottleneck – BATCH PLUS Simulation Flowsheet



Solution Without Bottleneck – Stream Variables

Step Stream Table

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 12/18/01 13:26

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 2.24 kg

BATCH PLUS Stream Label			1.1. Charge-1	1.1. Charge-2	1.4. Microfilter-6	1.4. Microfilter-7	1.4. Microfilter-8	1.5. Charge-11
Operation			1.1. Charge	1.1. Charge	1.4. Microfilter	1.4. Microfilter	1.4. Microfilter	1.5. Charge
Start Time		(min)	0.00	0.00	4,380.00	4,380.00	4,380.00	4,500.00
End Time		(min)	60.00	60.00	4,500.00	4,500.00	4,500.00	4,560.00
Total Time		(min)	60.00	60.00	120.00	120.00	120.00	60.00
From Unit					Mixing Tank	Microfilter	Microfilter	
To Unit			Mixing Tank	Mixing Tank	Microfilter	Mixing Tank	Holding Tank 1	Fermenter 1
Stream Type			Input	Input	Intermediate	Intermediate	Intermediate	Input
Mass - (kg)	**Per Batch**	**Mol Wt**						
Total			458.3000	3,565.0000	4,023.3000	4,023.3000	4,023.3000	1.2000
Endotoxin		18.02						
tPA		18.02						
AIR		28.95						
CARBON-DIOXIDE		44.01						
WATER		18.02		3,565.0000	3,565.0000	3,565.0000	3,565.0000	
Media		18.02	458.3000		458.3000	458.3000	458.3000	
tPA-CHO Cells		18.02						1.2000
Total Mass		(kg)	458.30	3,565.00	4,023.30	4,023.30	4,023.30	1.20
Total Volume		(liter)	461.15	3,587.19	3,968.36	3,968.36	3,968.36	1.21
Mass Flowrate		(kg/min)	7.64	59.42	33.53	33.53	33.53	0.02
Volume Flowrate		(liter/h)	461.15	3,587.19	1,984.18	1,984.18	1,984.18	1.21
Composite Product Factor			204.44	1,590.30	1,794.74	1,794.74	1,794.74	0.54
Phase			Liquid1	Liquid1	Liquid1	Liquid1	Liquid1	Liquid1
Temperature		(C)	25.00	25.00	4.00	4.00	4.00	25.00
Average Density		(kg/Cubic m)	993.81	993.81	1,013.84	1,013.84	1,013.84	993.81
Average Viscosity		(cp)	0.92	0.92	1.53	1.53	1.53	0.92
Average Heat Capacity		(kJ/kg-K)	4.18	4.18	4.21	4.21	4.21	4.18
Average Molecular Weight			18.02	18.02	18.02	18.02	18.02	18.02

Solution Without Bottleneck – Stream Variables (Cont'd.)

Step Stream Table

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 12/18/2001 13:26

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 2.24 kg

BATCH PLUS Stream Label			1.6. Transfer-Through-Heat-Exchanger-13	1.6. Transfer-Through-Heat-Exchanger-14	1.7. Ferment-18	1.7. Ferment-19	1.8. Transfer-21	1.12. Clean-24
Operation			1.6. Transfer-Through-Heat-Exchanger	1.6. Transfer-Through-Heat-Exchanger	1.7. Ferment	1.7. Ferment	1.8. Transfer	1.12. Clean
Start Time		**(min)**	4,560.00	4,560.00	5,280.00	5,280.00	12,480.00	13,200.00
End Time		**(min)**	5,280.00	5,280.00	5,280.30	5,286.76	13,200.00	13,201.00
Total Time		**(min)**	720.00	720.00	0.30	6.76	720.00	1.00
From Unit			Holding Tank 1	Heat Exchanger 1			Fermenter 1	
To Unit			Heat Exchanger 1	Fermenter 1	Fermenter 1	Fermenter 1	Fermenter 2	Fermenter 1
Stream Type			Intermediate	Intermediate	Input	Input	Intermediate	Input
Mass - (kg)	**Per Batch**	**Mol Wt**						
Total			21.2000	21.2000	0.0200	0.3000	22.4000	60.0000
Endotoxin		18.02					0.0022	
tPA		18.02					0.0022	
AIR		28.95				0.3000		
CARBON-DIOXIDE		44.01			0.0200			
WATER		18.02	18.7851	18.7851			18.9690	60.0000
Media		18.02	2.4149	2.4149				
tPA-CHO Cells		18.02					3.4265	
Total Mass		**(kg)**	21.20	21.20	0.02	0.30	22.40	60.00
Total Volume		**(liter)**	20.91	21.58	11.12	253.53	22.73	60.37
Mass Flowrate		**(kg/min)**	0.03	0.03	0.07	0.04	0.03	37.27
Volume Flowrate		**(liter/h)**	1.74	1.80	2,250.00	2,250.00	1.89	2,250.00
Composite Product Factor			9.46	9.46	0.01	0.13	9.99	26.77
Phase			Liquid1	Liquid1	Gas	Gas	Liquid1+Solid	Liquid1
Temperature		**(C)**	4.01	37.00	25.00	25.00	36.31	25.00
Average Density		**(kg/Cubic m)**	1,013.84	982.17	1.80	1.18	985.41	993.81
Average Viscosity		**(cp)**	1.53	0.71	0.01	0.02	0.61	0.92
Average Heat Capacity		**(kJ/kg-K)**	4.21	4.17	0.85	1.00	4.17	4.18
Average Molecular Weight			18.02	18.02	44.01	28.95	18.02	18.02

Solution Without Bottleneck – Stream Variables (Cont'd.)

Step Stream Table

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 12/18/2001 13:26

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 2.24 kg

BATCH PLUS Stream Label			1.12. Clean-25	1.9. Transfer-Through-Heat-Exchanger-26	1.9. Transfer-Through-Heat-Exchanger-27	1.10. Ferment-33	1.10. Ferment-34	1.11. Transfer-36
Operation			1.12. Clean	1.9. Transfer-Through-Heat-Exchanger	1.9. Transfer-Through-Heat-Exchanger	1.10. Ferment	1.10. Ferment	1.11. Transfer
Start Time		(min)	14,401.00	13,200.00	13,200.00	13,920.00	13,920.00	24,000.00
End Time		(min)	14,402.00	13,920.00	13,920.00	13,925.93	14,021.41	24,097.55
Total Time		(min)	1.00	720.00	720.00	5.93	101.41	97.55
From Unit			Fermenter 1	Holding Tank 1	Heat Exchanger 1			Holding Tank 1
To Unit				Heat Exchanger 1	Fermenter 2	Fermenter 2	Fermenter 2	Holding Tank 2
Stream Type			Output	Intermediate	Intermediate	Input	Input	Intermediate
Mass - (kg)	**Per Batch**	**Mol Wt**						
Total			60.0000	293.5000	293.5000	0.4000	4.5000	3,708.6000
Endotoxin		18.02						
tPA		18.02						
AIR		28.95					4.5000	
CARBON-DIOXIDE		44.01				0.4000		
WATER		18.02	60.0000	260.0670	260.0670			3,286.1479
Media		18.02		33.4330	33.4330			422.4521
tPA-CHO Cells		18.02						
Total Mass		(kg)	60.00	293.50	293.50	0.40	4.50	3,708.60
Total Volume		(liter)	60.37	289.49	298.83	222.36	3,802.88	3,657.98
Mass Flowrate		(kg/min)	37.27	0.41	0.41	0.07	0.04	38.02
Volume Flowrate		(liter/h)	2,250.00	24.12	24.90	2,250.00	2,250.00	2,250.00
Composite Product Factor			26.77	130.93	130.93	0.18	2.01	1,654.36
Phase			Liquid1	Liquid1	Liquid1	Gas	Gas	Liquid1
Temperature		(C)	25.00	4.01	37.00	25.00	25.00	4.01
Average Density		(kg/Cubic m)	993.81	1,013.84	982.17	1.80	1.18	1,013.84
Average Viscosity		(cp)	0.92	1.53	0.71	0.01	0.02	1.53
Average Heat Capacity		(kJ/kg-K)	4.18	4.21	4.17	0.85	1.00	4.21
Average Molecular Weight			18.02	18.02	18.02	44.01	28.95	18.02

Solution Without Bottleneck – Stream Variables (Cont'd.)

Step Stream Table

Process (Version): Reactor Chain Debottlenecking (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 10/25/2001 11:12

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 2.01 kg

BATCH PLUS Stream Label			1.11. Transfer-32	2.1. Transfer-35	2.2. Transfer-Through-Heat-Exchanger-38	2.2. Transfer-Through-Heat-Exchanger-39	2.3. Ferment-43
Operation			1.11. Transfer	2.1. Transfer	2.2. Transfer-Through-Heat-Exchanger	2.2. Transfer-Through-Heat-Exchanger	2.3. Ferment
Start Time		(min)	24,000.00	24,097.59	24,817.59	24,817.59	26,977.59
End Time		(min)	24,097.59	24,817.59	26,977.59	26,977.59	27,059.12
Total Time		(min)	97.59	720.00	2,160.00	2,160.00	81.53
From Unit			Holding Tank 1	Fermenter 2	Holding Tank 2	Heat Exchanger 2	
To Unit			Holding Tank 2	Fermenter 3	Heat Exchanger 2	Fermenter 3	Fermenter 3
Stream Type			Intermediate	Intermediate	Intermediate	Intermediate	Input
Mass - (kg)	**Per Batch**	**Mol Wt**					
Total			3,710.3000	315.9000	3,710.3000	3,710.3000	5.5000
tPA		18.02		0.1263			
AIR		28.95					
CARBON-DIOXIDE		44.01					5.5000
WATER		18.02	3,286.2657	277.8808	3,286.2657	3,286.2657	
Media		18.02	424.0343		424.0343	424.0343	
tPA-CHO Cells		18.02		37.8928			
Total Mass		(kg)	3,710.30	315.90	3,710.30	3,710.30	5.50
Total Volume		(liter)	3,659.66	321.61	3,659.69	3,777.67	3,057.46
Mass Flowrate		(kg/min)	38.02	0.44	1.72	1.72	0.07
Volume Flowrate		(liter/h)	2,250.00	26.80	101.66	104.94	2,250.00
Composite Product Factor			1,844.00	157.00	1,844.00	1,844.00	2.73
Phase			Liquid1	Liquid1	Liquid1	Liquid1	Gas
Temperature		(C)	4.01	36.90	4.02	37.00	25.00
Average Density		(kg/Cubic m)	1,013.84	982.26	1,013.83	982.17	1.80
Average Viscosity		(cp)	1.53	0.72	1.53	0.71	0.01
Average Heat Capacity		(kJ/kg-K)	4.21	4.17	4.21	4.17	0.85
Average Molecular Weight			18.02	18.02	18.02	18.02	44.01

Solution Without Bottleneck – Stream Variables (Con't)

Step Stream Table

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 12/18/2001 13:26

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 2.24 kg

BATCH PLUS Stream Label			2.1. Transfer-39	2.4. Clean-42	2.4. Clean-43	2.2. Transfer-Through-Heat-Exchanger-44	2.2. Transfer-Through-Heat-Exchanger-45	2.3. Ferment-51
Operation			2.1. Transfer	2.4. Clean	2.4. Clean	2.2. Transfer-Through-Heat-Exchanger	2.2. Transfer-Through-Heat-Exchanger	2.3. Ferment
Start Time		(min)	24,097.55	24,817.55	26,018.55	24,817.55	24,817.55	26,977.55
End Time		(min)	24,817.55	24,818.55	26,019.55	26,977.55	26,977.55	28,555.04
Total Time		(min)	720.00	1.00	1.00	2,160.00	2,160.00	1,577.49
From Unit			Fermenter 2		Fermenter 2	Holding Tank 2	Heat Exchanger 2	
To Unit			Fermenter 3	Fermenter 2		Heat Exchanger 2	Fermenter 3	Fermenter 3
Stream Type			Intermediate	Input	Output	Intermediate	Intermediate	Input
Mass - (kg)	**Per Batch**	**Mol Wt**						
Total			315.9000	600.0000	600.0000	3,708.6000	3,708.6000	70.0000
Endotoxin		18.02	0.0024					
tPA		18.02	0.1232					
AIR		28.95						70.0000
CARBON-DIOXIDE		44.01						
WATER		18.02	278.8288	600.0000	600.0000	3,286.1479	3,286.1479	
Media		18.02				422.4521	422.4521	
tPA-CHO Cells		18.02	36.9456					
Total Mass		(kg)	315.90	600.00	600.00	3,708.60	3,708.60	70.00
Total Volume		(liter)	320.94	603.73	603.73	3,658.01	3,775.94	59,155.97
Mass Flowrate		(kg/min)	0.44	37.27	37.27	1.72	1.72	0.04
Volume Flowrate		(liter/h)	26.75	2,250.00	2,250.00	101.61	104.89	2,250.00
Composite Product Factor			140.92	267.65	267.65	1,654.36	1,654.36	31.23
Phase			Liquid1+Solid	Liquid1	Liquid1	Liquid1	Liquid1	Gas
Temperature		(C)	36.90	25.00	25.00	4.02	37.00	25.00
Average Density		(kg/Cubic m)	984.29	993.81	993.81	1,013.83	982.17	1.18
Average Viscosity		(cp)	0.63	0.92	0.92	1.53	0.71	0.02
Average Heat Capacity		(kJ/kg-K)	4.17	4.18	4.18	4.21	4.17	1.00
Average Molecular Weight			18.02	18.02	18.02	18.02	18.02	28.95

Solution Without Bottleneck – Stream Variables (Con't)

Step Stream Table

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 12/18/2001 13:26

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 2.24 kg

BATCH PLUS Stream Label			2.3. Ferment-52	2.6. Transfer-Through-Heat-Exchanger-54	2.6. Transfer-Through-Heat-Exchanger-55	2.7. Clean-59	2.7. Clean-60
Operation			2.3. Ferment	2.6. Transfer-Through-Heat-Exchanger	2.6. Transfer-Through-Heat-Exchanger	2.7. Clean	2.7. Clean
Start Time		(min)	26,977.55	38,497.55	38,497.55	39,937.55	44,018.55
End Time		(min)	27,059.08	39,937.55	39,937.55	39,938.55	44,019.55
Total Time		(min)	81.53	1,440.00	1,440.00	1.00	1.00
From Unit				Fermenter 3	Cooler		Fermenter 3
To Unit			Fermenter 3	Cooler	Centrifuge Holding Tank	Fermenter 3	
Stream Type			Input	Intermediate	Intermediate	Input	Output
Mass - (kg)	**Per Batch**	**Mol Wt**					
Total			5.5000	4,024.5000	4,024.5000	600.0000	600.0000
Endotoxin		18.02		0.0031	0.0031		
tPA		18.02		2.2417	2.2417		
AIR		28.95					
CARBON-DIOXIDE		44.01	5.5000				
WATER		18.02		3,565.0896	3,565.0896	600.0000	600.0000
Media		18.02					
tPA-CHO Cells		18.02		457.1656	457.1656		
Total Mass		(kg)	5.50	4,024.50	4,024.50	600.00	600.00
Total Volume		(liter)	3,057.46	4,089.08	3,975.82	603.73	603.73
Mass Flowrate		(kg/min)	0.07	2.79	2.79	37.27	37.27
Volume Flowrate		(liter/h)	2,250.00	170.38	165.66	2,250.00	2,250.00
Composite Product Factor			2.45	1,795.28	1,795.28	267.65	267.65
Phase			Gas	Liquid1+Solid	Liquid1+Solid	Liquid1	Liquid1
Temperature		(C)	25.00	36.93	4.00	25.00	25.00
Average Density		(kg/Cubic m)	1.80	984.21	1,012.24	993.81	993.81
Average Viscosity		(cp)	0.01	0.63	1.36	0.92	0.92
Average Heat Capacity		(kJ/kg-K)	0.85	4.17	4.21	4.18	4.18
Average Molecular Weight			44.01	18.02	18.02	18.02	18.02

Solution Without Bottleneck – Mixing Tank Report

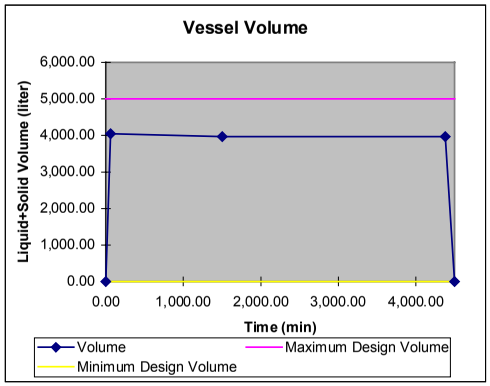
Step Equipment Contents

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 12/18/2001 13:26

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 2.24 kg

Mixing Tank

Operation		START	1.1. Charge	1.2. Cool	1.3. Age	1.4. Microfilter
Time	(min)	0.00	60.00	1,500.00	4,380.00	4,500.00
Mass - (kg)	Mol Wt					
Total		5.8957	4,024.4221	4,024.6086	4,024.6086	6.3424
WATER	18.02		3,565.0000	3,565.0000	3,565.0000	
NITROGEN	28.01	4.5223	0.8607	1.0038	1.0038	4.8650
OXYGEN	32.00	1.3734	0.2614	0.3048	0.3048	1.4774
Media	18.02		458.3000	458.3000	458.3000	
Liquid+Solid Mass	(kg)	0.00	4,023.30	4,023.30	4,023.30	0.00
Liquid+Solid Volume	(liter)	0.00	4,048.34	3,968.36	3,968.36	0.00
Phase		Gas	Gas+Liquid1	Gas+Liquid1	Gas+Liquid1	Gas
Temperature	(C)	25.00	25.00	4.00	4.00	4.00
Pressure	(kPa)	101.33	101.33	101.33	101.33	101.33
Average Liq+Sol Density	(kg/Cubic m)	0.00	993.81	1,013.84	1,013.84	0.00
Average Liq+Sol Viscosity	(cp)	0.00	0.92	1.53	1.53	0.00
Average Liq+Sol Heat Capacity	(kJ/kg-K)	0.00	4.18	4.21	4.21	0.00
Average Liq+Sol Molecular Weight		0.00	18.02	18.02	18.02	0.00



Solution Without Bottleneck – Holding Tank 2 Report

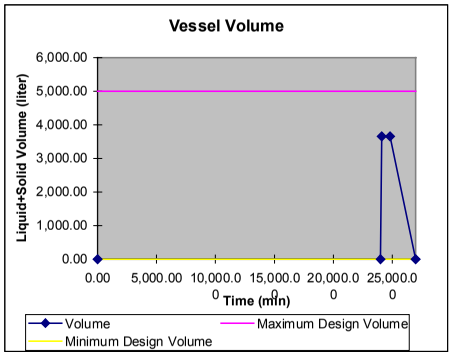
Step Equipment Contents

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 12/18/2001 13:26

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 2.24 kg

Holding Tank 2

Operation		START	1.11. Transfer	1.11. Transfer	2.2. Transfer-Through-Heat-Exchanger	2.2. Transfer-Through-Heat-Exchanger
Time	**(min)**	0.00	24,000.00	24,097.55	24,817.55	26,977.55
Mass - (kg)	**Mol Wt**					
Total		5.8957	5.8957	3,710.3022	3,710.3022	6.3421
WATER	18.02			3,286.1479	3,286.1479	
NITROGEN	28.01	4.5223	4.5223	1.3057	1.3057	4.8647
OXYGEN	32.00	1.3734	1.3734	0.3965	0.3965	1.4774
Media	18.02			422.4521	422.4521	
Liquid+Solid Mass	**(kg)**	0.00	0.00	3,708.60	3,708.60	0.00
Liquid+Solid Volume	**(liter)**	0.00	0.00	3,658.01	3,658.01	0.00
Phase		Gas	Gas	Gas+Liquid1	Gas+Liquid1	Gas
Temperature	**(C)**	25.00	25.00	4.02	4.02	4.02
Pressure	**(kPa)**	101.33	101.33	101.33	101.33	101.33
Average Liq+Sol Density	**(kg/Cubic m)**	0.00	0.00	1,013.83	1,013.83	0.00
Average Liq+Sol Viscosity	**(cp)**	0.00	0.00	1.53	1.53	0.00
Average Liq+Sol Heat Capacity	**(kJ/kg-K)**	0.00	0.00	4.21	4.21	0.00
Average Liq+Sol Molecular Weight		0.00	0.00	18.02	18.02	0.00



Solution Without Bottleneck – Fermenter 1 Report

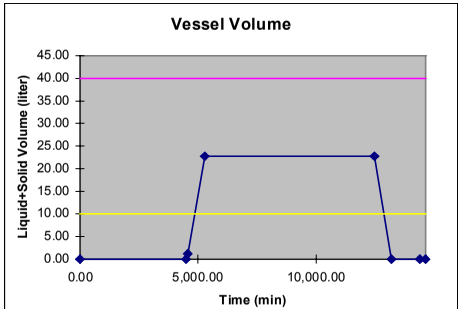
Step Equipment Contents

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 1/14/2002 18:26

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 2.24 kg

Fermenter 1

Operation		START	1.5. Charge	1.5. Charge	1.6. Transfer-Through-Heat-Exchanger	1.7. Ferment	1.8. Transfer	1.12. Clean
Time	**(min)**	0.00	4,500.00	4,560.00	5,280.00	12,480.00	13,200.00	14,402.00
Mass - (kg)	**Mol Wt**							
Total		0.0472	0.0472	1.2457	22.4195	22.4201	0.0459	0.0459
Endotoxin	18.02					0.0022		
tPA	18.02					0.0022		
AIR	28.95					0.0177	0.0177	0.0177
CARBON-DIOXIDE	44.01					0.0012	0.0012	0.0012
WATER	18.02				18.7851	18.9690		
NITROGEN	28.01	0.0362	0.0362	0.0351	0.0150	0.0009	0.0207	0.0207
OXYGEN	32.00	0.0110	0.0110	0.0107	0.0046	0.0003	0.0063	0.0063
Media	18.02				2.4149			
tPA-CHO Cells	18.02			1.2000	1.2000	3.4265		
Liquid+Solid Mass	**(kg)**	0.00	0.00	1.20	22.40	22.40	0.00	0.00
Liquid+Solid Volume	**(liter)**	0.00	0.00	1.21	22.79	22.73	0.00	0.00
Phase		Gas	Gas	Gas+Liquid1	Gas+Liquid1	Gas+Liquid1+Solid	Gas	Gas
Temperature	**(C)**	25.00	25.00	25.00	36.35	36.31	36.31	36.31
Pressure	**(kPa)**	101.33	101.33	101.33	101.33	101.33	101.32	101.32
Average Liq+Sol Density	**(kg/Cubic m)**	0.00	0.00	993.81	982.80	985.41	0.00	0.00
Average Liq+Sol Viscosity	**(cp)**	0.00	0.00	0.92	0.72	0.72	0.00	0.00
Average Liq+Sol Heat Capacity	**(kJ/kg-K)**	0.00	0.00	4.18	4.17	4.17	0.00	0.00
Average Liq+Sol Molecular Weight		0.00	0.00	18.02	18.02	18.02	0.00	0.00



Solution Without Bottleneck – Fermenter 2 Report

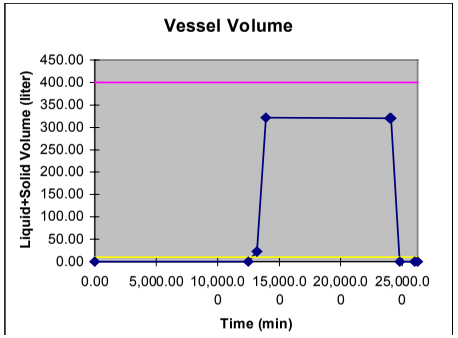
Step Equipment Contents

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 1/14/2002 18:26

Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 2.24 kg

Fermenter 2

Operation		START	1.8. Transfer	1.8. Transfer	1.9. Transfer-Through-Heat-Exchanger	1.10. Ferment	1.1. Transfer	1.1. Transfer	1.4. Clean
Time	**(min)**	0.00	12,480.00	13,200.00	13,920.00	24,000.00	24,097.55	24,817.55	26,019.55
Mass - (kg)	**Mol Wt**								
Total		0.4717	0.4717	22.8287	315.9889	315.9925	315.9925	0.4564	0.4564
Endotoxin	18.02			0.0022	0.0022	0.0024	0.0024		
tPA	18.02			0.0022	0.0022	0.1232	0.1232		
AIR	28.95					0.0834	0.0834	0.0834	0.0834
CARBON-DIOXIDE	44.01					0.0074	0.0074	0.0074	0.0074
WATER	18.02			18.9690	279.0360	278.8288	278.8288		
NITROGEN	28.01	0.3618	0.3618	0.3288	0.0682	0.0013	0.0013	0.2804	0.2804
OXYGEN	32.00	0.1099	0.1099	0.0999	0.0207	0.0004	0.0004	0.0852	0.0852
Media	18.02				33.4330				
tPA-CHO Cells	18.02			3.4265	3.4265	36.9456	36.9456		
Liquid+Solid Mass	**(kg)**	0.00	0.00	22.40	315.90	315.90	315.90	0.00	0.00
Liquid+Solid Volume	**(liter)**	0.00	0.00	22.73	321.56	320.94	320.94	0.00	0.00
Phase		Gas	Gas	Gas+Liquid1+Solid	Gas+Liquid1+Solid	Gas+Liquid1+Solid	Gas+Liquid1+Solid	Gas	Gas
Temperature	**(C)**	25.00	25.00	36.26	36.95	36.90	36.90	36.90	36.90
Pressure	**(kPa)**	101.33	101.33	101.33	101.33	101.33	101.33	101.33	101.33
Average Liq+Sol Density	**(kg/Cubic m)**	0.00	0.00	985.46	982.41	984.29	984.29	0.00	0.00
Average Liq+Sol Viscosity	**(cp)**	0.00	0.00	0.72	0.71	0.72	0.72	0.00	0.00
Average Liq+Sol Heat Capacity	**(kJ/kg-K)**	0.00	0.00	4.17	4.17	4.17	4.17	0.00	0.00
Average Liq+Sol Molecular Weight		0.00	0.00	18.02	18.02	18.02	18.02	0.00	0.00



Solution Without Bottleneck – Fermenter 3 Report

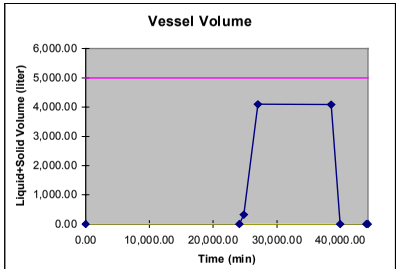
Step Equipment Contents

Process (Version): Reactor Growth Chain (1.0)
Step (Version): Step1 (1.0)
Simulation Date: 1/14/2002 18:26

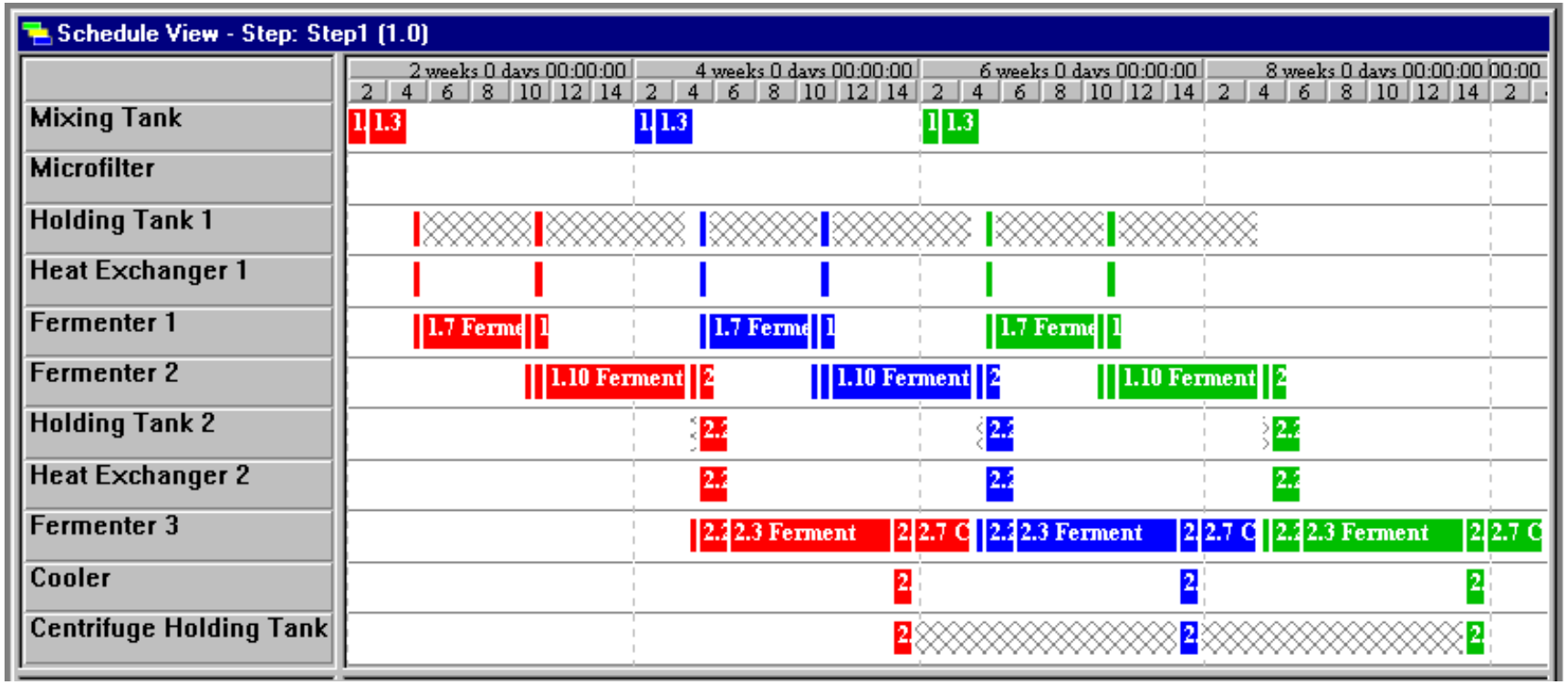
Key Input Intermediate: Media
Key Output Intermediate: tPA
Number of Batches: 1
Plan Quantity: 2.24 kg

Fermenter 3

Operation		START	1.1. Transfer	1.1. Transfer	1.2. Transfer-Through-Heat-Exchanger	1.3. Ferment	1.6. Transfer-Through-Heat-Exchanger	1.7. Clean
Time	**(min)**	0.00	24,097.55	24,817.55	26,977.55	38,497.55	39,937.55	44,019.55
Mass - (kg)	**Mol Wt**							
Total		5.8957	5.8957	321.2064	4,025.5238	4,025.5625	5.6985	5.6985
Endotoxin	18.02			0.0024	0.0024	0.0031		
tPA	18.02			0.1232	0.1232	2.2417		
AIR	28.95					0.9719	0.9719	0.9719
CARBON-DIOXIDE	44.01					0.0764	0.0764	0.0764
WATER	18.02			278.8288	3,564.9768	3,565.0896		
NITROGEN	28.01	4.5223	4.5223	4.0703	0.7853	0.0109	3.5670	3.5670
OXYGEN	32.00	1.3734	1.3734	1.2361	0.2385	0.0033	1.0833	1.0833
Media	18.02				422.4521			
tPA-CHO Cells	18.02			36.9456	36.9456	457.1656		
Liquid+Solid Mass	**(kg)**	0.00	0.00	315.90	4,024.50	4,024.50	0.00	0.00
Liquid+Solid Volume	**(liter)**	0.00	0.00	320.93	4,096.86	4,089.08	0.00	0.00
Phase		Gas	Gas	Gas+Liquid1+Solid	Gas+Liquid1+Solid	Gas+Liquid1+Solid	Gas	Gas
Temperature	**(C)**	25.00	25.00	36.85	36.99	36.93	36.93	36.93
Pressure	**(kPa)**	101.33	101.33	101.33	101.33	101.33	101.32	101.32
Average Liq+Sol Density	**(kg/Cubic m)**	0.00	0.00	984.34	982.34	984.21	0.00	0.00
Average Liq+Sol Viscosity	**(cp)**	0.00	0.00	0.72	0.71	0.72	0.00	0.00
Average Liq+Sol Heat Capacity	**(kJ/kg-K)**	0.00	0.00	4.17	4.17	4.17	0.00	0.00
Average Liq+Sol Molecular Weight		0.00	0.00	18.02	18.02	18.02	0.00	0.00



Solution Without Bottleneck – Multiple Batch Gantt Chart



Solution Without Bottleneck – Single Batch Gantt Chart