Computer-Aided Design of Integrated Biochemical Processes; Development of BioDesigner

by

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ABSTRACT

A new systematic design methodology has been formulated and implemented on the computer that can assist the designer of large scale integrated biochemical processes.

The design methodology is hierarchical because it decomposes the problem of developing and evaluating a flowsheet for the production of a biological product into a number of easier subproblems or design levels. At design level 1, the input-output structure of the flowsheet is considered and the main objective is to estimate the amount and cost of raw materials required for the production of a certain amount of product and by-products. At level 2, the entire flowsheet is decomposed into two sections, upstream and downstream, and design efforts are focused on the upstream section. The main tasks at this level are to synthesize the upstream section (select the type of biocatalyst, the type of bioreactor, etc.), estimate the size of equipment, estimate the production cost of upstream section, and carry out an economic evaluation. At level 3, design efforts are focused on the downstream section. The main tasks at this level are to synthesize the downstream section, complete the material and energy balances for the entire flowsheet, estimate the equipment size and capital cost, estimate the production cost, and carry out an economic evaluation of the entire process.

In this methodology, the complete flowsheet is considered at each design level but more detailed structure is added at each hierarchy of
design. Heuristics or rules of thumb are used to fix flowsheet structure, impose constraints, and estimate critical design parameters.

BioDesigner is the program that implements on the computer the above design methodology. It consists of three components: the user interface, the synthetic component, and the analytic component. The analytic component is written in C and, for a given flowsheet, estimates the material and energy balances and carries out an economic evaluation. The synthetic component consists of two knowledge bases which along with the inference engine of Nexpert Object synthesize flowsheets for biochemical processes. BioDesigner features a highly interactive and user friendly interface that assists process engineers to do their work more efficiently.

BioDesigner has been used to carry out a number of case studies for a variety of biological products, including high value proteins, biopolymers, and antibiotics. The results evaluated by professionals were found realistic. BioDesigner has been used to assist in designing experimental protocols for pilot-plant production of porcine Somatotropin. BioDesigner has made its first steps into education. It has been used by students to carry out term design projects in two graduate courses (biochemical separations and biochemical engineering) in the Department of Chemical Engineering at MIT.

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Professor of Chemical and Biochemical Engineering

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Professor of Chemical Engineering
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DEDICATION

To my wife, Elpida.
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Chapter 1

Introduction

The recent advances in biotechnology have increased the need for large-scale production of many new biological materials. As a consequence, there is increased interest in formulating design methodologies and developing software tools that can facilitate process design and development of biochemical processes.

Such methodologies and tools can be used to: 1) screen project ideas and focus on the most promising ones; 2) improve communication between the various groups involved in process development; 3) design experimental protocols, interpret experimental results, and reduce the time required for process development; 4) design more robust processes; 5) optimize the operation of existing facilities and minimize their production cost.

The development of such methodologies and tools, however, presents a difficult problem to biochemical engineers because: 1) there is a large diversity of biochemical products widely differing in physical and chemical properties; 2) most biological products, especially the high value ones, are complex and labile materials (e.g., proteins and polysaccharides), often required in very high purity, and their value depends on functionality and not on mass; 3) most biological products are poorly characterized with respect to physical properties (e.g., viscosity, diffusivity, specific heat, solubility, etc.) and these values are difficult to predict from thermodynamic and microtransport models; 4) several bioprocess unit operations are poorly understood and predictive models which could be
used for design do not exist; 5) most bioprocesses operate in batch or semicontinuous mode introducing time as an independent variable;

Despite these difficulties, the scientific challenge and the potential reward from the commercialization of biotechnology has stimulated interest in recent years in developing process analysis (Evans and Field, 1988) and process synthesis tools (Silletti, 1988) for biochemical processes.

Process design and development requires a combination of synthesis and analysis steps and the challenge is to efficiently integrate these two activities in process design methodologies and tools. Such methodologies and tools must be able to utilize all forms of available knowledge, both algorithmic and experiential. Algorithmic knowledge, mainly in the form of short-cut models for the various unit operations, is useful in carrying out the analytic tasks of design (for instance, calculation of material and energy balances). Experiential knowledge, mainly in the form of heuristics and rules of thumb, is useful in carrying out the synthetic tasks of design. They must offer to biochemical engineers the flexibility to synthesize and analyze interactively a large number of project ideas in a short time. Finally, they must be easy to use even for occasional users.

**Thesis Objectives:** The overall objective of this thesis is to formulate a design methodology, implement it on the computer, and test its utility for process development in the field of biotechnology. The specific objectives are:

1. To formulate a systematic, design methodology that will assist the bioprocess engineer to efficiently carry out preliminary design of multiple integrated processes for manufacturing of biochemical products.
2. To implement the design methodology on the computer (BioDesigner is the name of the program).

3. To show how to efficiently combine synthetic with analytic process design tools, and

4. To show how to develop intelligent user interfaces for process design software that assist the engineer to quickly develop and analyze alternative designs.

**Organization:** Besides the present chapter, this dissertation contains eight additional chapters and four appendices. Chapter 2 presents a historical review on computer-aided design of entire flowsheets with emphasis on process synthesis and compares BioDesigner with previous software design tools. Chapter 3 describes the systematic design methodology that BioDesigner follows. Chapter 4 presents implementation features and the system architecture of BioDesigner. Chapter 5 describes how synthesis is done in BioDesigner. Chapter 6 describes how analysis is done in BioDesigner. Chapter 7 presents the unit operation models used in BioDesigner. Chapter 8 illustrates, through a case study, how BioDesigner would be used by a biotechnologist to design a flowsheet for the production of a high value product. Chapter 9 presents the conclusions and recommendations for future work. Appendix I constitutes the manual for BioDesigner. Appendix II presents the objects and production rules used in the knowledge bases of BioDesigner. Appendix III describes the macroscopic elemental balance facility of BioDesigner. Finally, appendix IV is the detailed stream report of the design case presented in Chapter 8.
Chapter 2

Historical Review

This chapter is not intended to be an exhaustive review of the literature on computer-aided process design. Rather, it will first present briefly the main research topics on computer-aided analysis and synthesis of entire chemical process flowsheets and then consider the work done on computer-aided design of biochemical processes.

2.1 Computer-Aided Process Analysis

Work in computer-aided process design started some 30 years ago (see Fig. 2.1). In the 1960's and most of the 1970's, research focused on the analytic aspects of design and resulted in the development of powerful process simulators for steady-state processes, such as FLOWTRAN developed at Monsanto (Rosen and Pauls, 1977) and ASPEN developed in the department of Chemical Engineering at MIT (Evans et. al., 1979). In the 80's, there was development of dynamic process simulators, such as SPEED-UP developed at Imperial College in England (Perkins and Sargent, 1982; Pantelides, 1988).

Figure 2.2 shows the main components of a process simulator. The heart of any process simulator is the ability to model the performance of unit operations that compose a flowsheet. A great deal of work has been
Figure 2.1: Previous work in computer-aided process design
Figure 2.2: Main modules of a process simulator
done over the past few decades to develop rigorous models for unit operations typically found in the more traditional segments of the chemical process industries (Boston and Britt, 1978). These models describe complicated equilibrium phenomena, reaction systems, and energy balances often encountered in chemical systems. Because most chemical processes are generally operated continuously and at steady-state, the equations representing the material and energy balances are usually non-linear and algebraic in nature. On the other hand in biochemical processes, batch and semicontinuous processes are the rule rather than the exception. This makes rigorous simulation of biochemical processes a considerably more difficult task because integration of systems of differential equations is required. To minimize program execution and software development times, short-cut models of biochemical unit operations were mainly used in BioDesigner (BioDesigner is the name of the program that was developed as a part of this thesis). This is not a serious handicap of BioDesigner because the so called rigorous models of biochemical processes are not, most of the times, more predictive than the short-cut ones.

The development of flowsheet simulators would have been impossible without suitable mathematical algorithms to solve the systems of algebraic or differential equations used to describe chemical processes or without the growth of inexpensive and powerful computers. The 1960's and 1970's saw a great amount of progress in the areas of algorithms and computer power. Sargent (1981) presented a review of algorithms for systems of non-linear algebraic equations. The increases in computer speeds and memory sizes have allowed more sophisticated algorithms to be applied to more complicated problems. The advent and exponential growth in power of
personal computers have moved process simulators from mainframe computers to the engineer's desk and have drastically reduced the cost of computer time.

The development of rigorous process simulators would have been impossible without the progress in thermodynamic and microtransport properties prediction methods. Such algorithms are required to estimate physical properties data to accurately describe equilibrium and kinetic phenomena in unit operations under various operating conditions. Unfortunately the situation is not as easy for biochemical processes. The complexity of many biomaterials makes development of appropriate physical properties prediction algorithms a very difficult task and this hinders development of rigorous bioprocess simulators.

Three main architectures are used in the development of process simulators: sequential modular, equation-oriented, and simultaneous modular (Chen and Stadtherr, 1985; Westerberg et al., 1979).

**Sequential Modular** is by far the most commonly used approach. Simulators following this approach have subroutines that model specific unit operations. These models are organized into unit operation modules which calculate unit output stream values given unit input stream values and equipment parameters. This approach is natural to the user because information flow follows flow of material in the streams. It is easier to implement and debug the software compared to the other two approaches. However, this approach is not efficient in handling complex processes with a large number of nested recycle loops. Also, it is not well suited to the solution of optimization problems because the entire flowsheet may have to be solved iteratively a large number of times before the optimum solution is found.
Equation-Oriented Approach. In this approach, the unit operation model equations, connection equations, and specifications are treated as a large system of nonlinear equations to be solved simultaneously. Since the equations are solved simultaneously, there is no need for iterative procedures to solve recycle loops or design specifications. Since the simultaneous equations can be used as constraints in a generalized nonlinear programming problem, this approach is well suited for process optimization. However, a number of problems have restricted the widespread industrial adoption of the equation-oriented approach. It is mathematically more demanding because it requires solution of large systems of nonlinear equations (thousands). The equation-oriented approach has acquired a poor reputation for reliability because of its frequent inability to successfully converge these large systems of equations for real world problems. Another problem is that the flexibility associated with the equation-oriented approach makes it easier for the user to make inconsistent specifications and error checking is harder.

Simultaneous Modular Approach. In this approach unit operation modules are written the same as for the sequential modular approach. Each module calculates the output stream values of a unit given its input stream information and equipment parameter values. At this point, there is a fundamental difference. A second module for each unit operation must be written to relate each output value approximately to a linear combination of all input values. These small sets of linear equations are then combined to form a larger set that describes the entire flowsheet. As in the equation-oriented approach, in the simultaneous modular approach the equations describing the entire process are solved simultaneously. This approach combines the advantages and drawbacks of the previous two techniques.
The most fundamental problems with the simultaneous modular approach involves the solution of the flowsheet level equations; this requires a general purpose nonlinear equation solver, typically the Newton-Raphson method or some variation thereof, and hence reliability can become a problem.

Most biochemical flowsheets have few (if any) recycle loops. Further, process robustness and not optimization is the main objective for most bioprocesses, especially the ones that deal with the production of high value products. Therefore, the use of sequential modular approach seems adequate at least for the development of first generation bioprocess simulation tools. BioDesigner follows this approach.

Most process simulators have models for estimating capital cost of equipment. They provide facilities to carry out economic evaluation and profitability analysis for a project idea.

Development of Intelligent Interfaces for process simulators has become a "must" in recent years. Users expect to spend hours and not days or weeks to learn how to use a new piece of process design software. The use of advanced graphics for flowsheet representation, windows, menus, mice, etc., find increasing use in design programs. Intelligent interfaces improve communication between the user and the computer and reduce the learning period by lifting the burden of learning obscure languages used in input files of conventional process simulators. Following this spirit, BioDesigner has been developed with a very user friendly and interactive interface. The program is fully event-driven and most actions that the user takes are self-explanatory. BioDesigner's interface is part of the program and not a separate application like the up-front interfaces that some companies have developed for existing commercial simulators. BioDesigner does not create and submit input files to run a simulation and
no time is wasted for that. More information on the interface of BioDesigner can be found in Chapter 4.

Process simulators are mainly used to analyze and evaluate complex processing systems, carry out sensitivity analysis, and optimize given flowsheets. They have been successful in minimizing production cost of existing plants and improving the design of new plants (Brannock et al., 1981; Proctor, 1983). These tools, however, lack any synthetic capabilities.

2.2 Computer-Aided Process Synthesis

Work in automation of synthesis of entire flowsheets began in the late 1960's with Prof. Rudd and his students at the Univ. of Winsconsin (Siirola and Rudd, 1971). Since then, several approaches and methodologies have been developed to solve the same problem with variable degree of success. The various approaches belong to one or more of the following categories:

- Heuristic-Algorithmic techniques
- Evolutionary design
- Branch and bound strategy
- Structural parameter approach
- Hierarchical approach
- Knowledge based approach

A brief review of these approaches follows.

**Heuristic-Algorithmic Techniques**

These techniques use a search algorithm to generate alternatives and then apply domain-specific heuristics to reduce the size of the search
space. AIDES (Adaptive Initial Design Synthesizer) is a pioneering work in this category (Siirola et al., 1971; Siirola and Rudd, 1971; Power, 1972). It was written in ALGOL and run on a Burroughs 5500. It used linked lists to represent internal data structures. Physical property information required by AIDES includes molecular weight, normal melting and boiling points, estimates of corrosiveness, and some solubility data. AIDES makes use of a modified version of the *means-ends analysis* technique; given information on raw materials and the desired products, it attempts to bridge the gap (between raw materials and product) through a sequence of operations that are eventually transformed into unit operations. Most heuristics applied by AIDES were for sharp separation sequencing and dealt with low molecular weight chemical compounds. Some examples of the heuristics include:

- *Remove the most plentiful component first*
- *Make the most difficult and most expensive separations last*
- *Remove any corrosive components early in the sequence*

Seven years after AIDES was developed, another program (BALTAZAR) combining heuristic and algorithmic techniques in process synthesis was developed (Mahalec and Motard, 1977a,b). BALTAZAR combines depth-first sequential search with some heuristics to produce feasible process structures. It also used the techniques for mechanical theorem proving. BALTAZAR was written in LISP and run on a UNIVAC 1108. It is interesting to note that neither of these two programs was written in a traditional engineering-oriented programming language like FORTRAN.

**Evolutionary Approaches**
The evolutionary approach starts with a previously synthesized flowsheet and seeks better structures (according to an objective function) through a sequence of modifications (Stephanopoulos and Westerberg, 1976; Seader and Westerberg, 1977; Nath and Motard, 1981). The four important steps in this approach are: (1) developing an initial flowsheet; (2) choosing rules for evolving the structure; (3) adopting an evolutionary strategy; and (4) using a method to evaluate the current flowsheet and its neighbors. The final result of this methodology depends strongly on the structure of the initial flowsheet. If only a few modifications are allowed at a time, this method may not be able to get from a local optimum to the global one. This approach usually appears as the second phase of some other technique, such as a heuristic technique, and has been applied mainly in the synthesis of separation systems.

**Branch and Bound Approach**

This approach makes use of an efficient search technique that eliminates large numbers of possible flowsheets by rejecting partial solutions that are more expensive than the best feasible flowsheet. It consists of two operations: (a) branching, which divides collections of solution sets into subsets and (b) bounding, which sets bounds on the value of the objective functions over subsets of solutions. This approach is practical because it eliminates many possibilities at once. This technique has found applications in the synthesis of separation systems of low molecular weight compounds (Westerberg and Stephanopoulos, 1975), (Gomez and Seader, 1976), and (Morari and Faith, 1980).

**Structural parameter method**
This approach combines all possible alternatives into one integrated "superstructure" in which all possible interconnections among process units are included. "Structural parameters" are variables that range between zero and one and represent stream splits. When a structural parameter equals zero, the flowsheet is connected one way; when it equals one, the flowsheet is connected the other way. When the structural parameter is between zero and one the flowsheet is connected both ways at once. The best structure is found by optimizing over the process models and structural parameters using nonlinear or mixed integer nonlinear programming techniques. Floudas (1987, 1988) employed this technique to obtain sequences of non-sharp separators. Floudas et al. (1986) employed the same technique to obtain optimal heat exchanger networks satisfying the criteria of minimum utility cost and minimum number of heat exchanger units. The potential configurations that are considered can include series and/or parallel arrangements as well as stream splitting, mixing, and bypassing. The difficulty of the optimization (computationally intensive) is the chief drawback of the structural parameter method.

Hierarchical - Heuristic Approach

This approach decomposes the problem of design into a number of design or decision levels sequenced and solved in a hierarchical order. It first sketches a design that is complete but vague, and then refines the vague parts into more detailed designs until finally the design has been refined to a complete sequence of detailed unit operations. Heuristics are used at all levels of decision to fix the structure of the flowsheet, impose design constraints, and substantially reduce the number of alternatives that have to be considered. This methodology, described best by Douglas
(1985, 1988) and Lu and Motard (1985), appears promising because the decisions are of the same type and in the same order as an engineer would naturally make them. A modified version of this methodology, adjusted to biochemical processes, has been implemented in BioDesigner.

**Artificial Intelligence Approaches**

An increasing number of researchers have applied artificial intelligence techniques to the process synthesis problem in the past few years. Knowledge Based Expert Systems (KBES) in particular seem most promising for applications to process synthesis. KBES offer a natural way to capture and document experiential design knowledge. Knowledge is stored in the form of production rules; an example of the typical syntax of such a rule is:

*IF the pH of the spill is less than 6,*

*THEN the spill material is an acid*

This methodology consists of storing domain-specific problem solving knowledge as independent declarative pieces of modular information in a way that leaves the control strategy of the overall program independent of it. The domain-specific knowledge is used to constrain the search space and select between alternative strategies. They are activated when their conditional part is satisfied by the current description of the design state space. The separability of control and domain-specific knowledge facilitates the incremental growth of the system.

STES (Separation Technology Expert System) by Naterfield and Sunol (1987) is implemented on ART (Automated Reasoning Tool, Inference
Corp.) on a Sun workstation. It is designed to facilitate the selection of separation techniques (mainly distillation sequences). STES appears to have limited capability owing to the fact that it solves only problems involving separation of one compound from a group of compounds. It is unclear how the system would handle separation of a group of compounds from another group of compounds.

OSSCAR (ORNLSeparator SCience Advisor) by Debelek et al. (1987) is implemented on OPS5 running under FranzLisp on a VAX 11/750. It has five control modules -- CORE, MAN-MACHINE, REGULATION, COORDINATION and SEPSYS. The mechanism governing the behavior of CORE is a planner comprising agenda. MAN-MACHINE is the control module that is the user interface. The REGULATION expert system determines which environmental regulations apply to the discharge. The COORDINATOR chooses one or several candidate separation techniques; the default is ion exchange. The SEPSYS (SEParation science SYStem data base) contains about 6000 references to work in separation science.

Most expert systems for process synthesis are developed using commercial expert systems shells. Expert system shells provide an inference engine which is an interpreter that executes the production rules that the user writes. They also provide an environment to develop knowledge bases. Several expert system shells provide "Frame" or "Object" representation facilities (Object Oriented Programming environments). Frames provide better organization of declarative knowledge. In this framework, information is organized into a taxonomy of classes. Each class can be described as a specialization (subclass) of other more generic classes. For example, the class of separators can be described as a specialization of the class of unit operations; in turn, the class of filters can
be described as a specialization of the class of separators. In fully Object
Oriented Programming environments, an object has some properties and
exhibits some behavior (Stefik and Bobrow, 1985; Stroustrup, 1988). The
merits of incorporating the notion of object in software programming are
well recognized; they facilitate achievement of data abstraction,
information hiding, dynamic binding, inheritance, fast prototyping,
software modularization, and software maintenance.

DESIGN-KIT is an object-oriented environment for process
engineering (Stephanopoulos et al., 1987). It runs on Symbolics, uses the
KEE expert system shell in combination with Common Lisp, and makes
use of the "Flavors" system for object-oriented programming. It provides an
advanced user interface and allows the user to build complex unit operation
models based on more primitive ones.

KBES are suitable to handle experiential knowledge but do not offer the
best environment to handle algorithmic and "number crunching" tasks.
Conventional programming languages are more appropriate for those
tasks. Further, high quality software for number crunching applications is
already available in conventional languages. To combine the best of the two
approaches, hybrid systems are often developed. In such systems, a KBES
that handles synthetic tasks is interfaced to a number crunching program.
The KBES is the main application and the number crunching program acts
as an external subroutine. However, the reverse also may be selected; one
can add intelligence to a number crunching program by interfacing it to a
KBES. This is facilitated by some expert system shells that are available in
more than one versions, namely "development", "runtime", etc. Runtime
versions are usually appropriate for embedding into other applications and
developing hybrid tools that can handle both synthetic and analytic tasks.
BioDesigner follows the latter approach. The analytic component that carries out all the "number crunching" tasks (e.g., calculation of material and energy balances) is the main application and is written in the C programming language. To carry out synthesis, the dynamic runtime library of Nexpert Object (a commercial expert system shell from Neuron Data, Inc.) has been embedded in BioDesigner.

2.3 Computer-Aided Bioprocess Design

Substantial research and development efforts in computer-aided design of integrated biochemical processes started in the mid 1980's following the development efforts and commercialization of new high value bioproducts. There are a number of difficulties which have delayed application of such advanced design techniques to bioprocesses. First, bioprocess industries make use of several unit operations not typically found in the traditional chemical process industries (e.g. chromatography, membrane filtration). Some of these unit operations are poorly understood and predictive mathematical models are absent. Second, most biological products are complex materials and methods to estimate their physical properties are not adequately developed. Complicated solution chemistry and the presence of solids in the process cause additional simulation difficulties. Third, the prevalence of batch and semi-continuous processing units in biochemical processes causes problems for flowsheeting. Batch operations are inherently time-dependent, which means that differential equations must be integrated. Although techniques are available to solve these systems of algebraic and differential equations, interfacing time-
dependent units with a program structure that assumes steady-state operation and the addition of discrete state variables make the simulation problem considerably more difficult.

**BioProcess Simulator (BPS).** BPS is an extension of the Aspen Plus process simulator (Evans and Field, 1988). Its development was initiated as a collaboration between AspenTech and MIT and was completed by AspenTech. BPS uses the infrastructure and the facilities provided by Aspen Plus. BPS, for a given flowsheet, carries out material and energy balances, estimates the size and cost of equipment, and carries out an economic evaluation. It is a steady-state simulator but can also handle time-dependent processes with some limitations. Batch processes, that require integration over time, are connected with the rest of the flowsheet through infinite intermediate storage capacity. The differential equations that describe a batch fermentor, for example, are integrated over fermentation time. At the end of fermentation, broth is transferred to a hypothetical tank before it starts being processed in the downstream section.

**BioSep Designer** (Siletti, 1988). BioSep Designer is a Knowledge Based system that carries out synthesis of protein separation systems. It is written in Common Lisp and runs on the Symbolics. It uses the "Flavors" system to implement Object-Oriented Programming. Based on a given set of input data, BioSep Designer generates possible flowsheets and employs an evaluation technique to estimate the optimum one. Its synthetic knowledge base contains information mainly focused on the production of proteins by *E.coli*. It has facilities to estimate some protein properties from their amino acid sequence. It provides a simple database to store protein properties.
BioSep Designer also has some analytic and economic evaluation capabilities.

**BioDesigner** is a program developed as a part of the present thesis. It carries out synthesis using a KBES based on the Nexpert Object expert system shell. The knowledge utilized for process synthesis is based on the properties of the producing microorganism, the product, the contaminants, etc. The result of a synthesis session, which is a feasible flowsheet, is automatically transferred to the analytic component for analysis and evaluation.

The analytic component of BioDesigner is written in the C programming language. BioDesigner runs on the Apple Macintosh computer and has a user friendly interface. BioDesigner has a more expanded scope in comparison to BioSep Designer. It handles diverse biological products whereas BioSep Designer deals only with proteins and at its full capacity only with proteins produced by *E.coli*. BioDesigner also handles both the upstream and downstream sections of a flowsheet while BioSep Designer focuses primarily on the downstream section. Finally, BioDesigner has a more advanced analytic component (estimation of material and energy balances, economic evaluation, etc.). On the other hand, BioSep Designer has a more advanced synthesis infrastructure; it can generate, store, and evaluate multiple alternative flowsheets whereas BioDesigner only generates one reasonable solution at a time.
Chapter 3

Design Methodology

3.1 Introduction

Design is the creative process that leads from the identification of a need to an end product, system, or process that satisfies that need. In the process industries, a new product could be a new solvent or a second generation antibiotic with certain properties; a new system device for manufacturing and a process could be a new facility for the production of a chemical or biochemical product. Process design consists of two main subactivities, process synthesis and process analysis.

Process Synthesis is the selection and arrangement of a set of unit operations capable of producing the desired product at an acceptable cost and quality.

Process Analysis is the analysis and comparison of solutions to the problem of product synthesis.

Process design is difficult because:

1) The design resources change with time as a consequence of changes in technology.

2) The design goals change with time because of changes in market, technology, etc.

3) It requires integration of knowledge from many different science and engineering disciplines; it also requires different types of
knowledge. For instance, synthesis requires experiential knowledge and analysis requires algorithmic knowledge.

4) There is no single design methodology enabling engineers to expeditiously transform new ideas into competitive products and processes.

**Biochemical process design** is as an extension of chemical process design with some unique features that contribute to its complexity.

*Products:* Most biological products are complex materials, such as proteins and polysaccharides. They are labile, often required in high purity (for instance, when used as parenteral drugs), and their value depends on functionality. Most are poorly characterized with respect to physical properties, such as viscosity, diffusivity, specific heat, solubility, etc., and these values are difficult to predict from thermodynamic or microtransport models.

*Processes:* Many bioprocess unit operations are available only for bench scale, operate in batch or semicontinuous mode and require regular maintenance and cleaning. These factors introduce time as an independent variable. In addition, product is typically found in low concentrations in a complex aqueous chemical environment. This renders the recovery and purification of many biological products a difficult task.

**Need for an Efficient Preliminary Design Methodology.** Experience has shown that less than 5% of new project ideas are ever commercialized (Douglas, 1988). Further, design problems are frequently open-ended with alternative solutions of differing economic viability. Consequently, it is important for a process designer in the biochemical process industry to
follow a systematic and efficient design procedure that will assist in the assessment of new project ideas at a preliminary design stage. With a systematic design procedure, key design assumptions needing experimental validation are rapidly identified. It is possible to identify process steps with high cost and/or low yield and R&D can be focused to reduce product introduction time.

Many research efforts decompose the design problem into a number of subproblems in a top down hierarchical manner following Douglas's approach (Douglas, 1988). BioDesigner's design methodology also follows the same philosophy. However, because of differences between chemical and biochemical processes the strategy and objectives of BioDesigner's methodology are different. For instance, selection of recycle loops and heat integration are very important for most petrochemical processes but not so important for biochemical processes because recycle loops are rarely used and small variation in stream temperatures reduces the benefits of heat integration. The ultimate objective of BioDesigner's methodology for the production of high value biological products is to select and arrange a set unit operations that are capable of achieving the required high product purity at an acceptable cost. On the other hand, for low value biological products, the main objective is to maximize product recovery.

This methodology can be used by students and engineers to efficiently develop a base case design for the production of a biological product. It shows how to decompose the complicated problems of bioprocess design into a number of easier subproblems and how to use experiential knowledge to carry out synthesis. Only when it is automated and implemented on the computer, however, this methodology does realize its potential as a powerful design tool. BioDesigner represents a successful implementation
of such a methodology. A description of the computer implementation can be found in Chapters 4, 5, and 6.

3.2 The Systematic Design Methodology

The problem of biochemical flowsheet design is decomposed into four subproblems or design levels sequenced in a hierarchical order.

At level 1, the input-output structure of the flowsheet is examined to estimate raw materials requirements. At level 2, the upstream section, including biocatalyst selection, bioreactor configuration, media preparation, sterilization, capital and operating cost are considered. At level 3, the downstream section is considered. Going through the first three levels, one makes many decisions and generates a base case design. When a clear-cut decision cannot be made, alternatives are generated. At design level 4, the level of optimization, alternatives are considered. In this thesis, only the first three design levels are considered.

In this methodology, the complete flowsheet is considered at each design level but more detailed structure is added at each hierarchy of design. Heuristics or rules of thumb are used to fix flowsheet structure, impose constraints, and estimate critical design parameters. Each design level requires data and results from the previous level along with new information and terminates with an economic assessment that determines project continuation.

LEVEL 1: INPUT - OUTPUT STRUCTURE OF THE FLOWSHEET
At this design level, the whole flowsheet is considered as a black box and the focus is on the input of raw materials and the output of product and by-products. For a specified plant capacity, the objective is to estimate requirement of raw materials. It is necessary to estimate product recovery in the downstream section because it affects raw materials requirement. At this design level, individual steps are not yet identified; it is necessary to estimate product production with one or more of the following approximations: 1) Stoichiometry, 2) Raw materials allocation model, 3) Analogy with similar products, 4) Laboratory data.

If the product biochemical pathway is known, a stoichiometric approximation can estimate product yield (Cooney and Avecedo, 1977). Such values constitute an upper bound to actual yield because they are not mechanistic; futile pathways, by-product formation, and maintenance are ignored.

To partially resolve this problem, an allocation model accounting for nonidealities is applied. Such a model is based on the simple concepts of: mass conservation, degree of reduction of chemical and biochemical compounds, and maintenance coefficients. A detailed description with data can be found in Roels (1983). This technique leads to the estimation of carbon and nitrogen requirements for biomass and product formation. The amount of oxygen also is estimated. Other components (P, S, Mg, Ca, K, Na, etc.) are usually supplied by various inorganic salts (see Table 3.1) and amounts estimated from an elemental balance for the system. Information on media composition and formulation is available in the
relevant literature (Cejka, 1985; Dimmling and Nesemann 1985; Zabriskie et al. 1982).

For protein production, it is not possible to discriminate between product and cell growth by stoichiometry. An alternative concept is needed. One approach is the *product expression level*. For proteins produced by most organisms, the protein expression level it typically <1%. However, with recombinant organisms it can be >30% of cell protein and 5% to 20% common. When data on expression are not available, a value is selected by analogy to similar products. Knowing the expression level and the amount of cell protein, typically 50-60% of cell mass, one can estimate the amount of biomass and raw materials required.

To facilitate the design at this first level, BioDesigner provides a facility to carry out an elemental balance around any reaction system. A detailed description of this facility is provided in Appendix III.

Not all of the product is recovered and three parameters mainly affect the recovery yield:

*Product lability*. Complex labile biomolecules may degrade during downstream processing resulting in low recovery yield.

*Initial product purity*. The initial product concentration as well as the type and concentration of impurities strongly affect the recovery yield. The lower the initial purity, the larger the number of recovery steps, resulting in low overall yield. Recovery yield of individual steps can vary from almost 100% to 50% with an average for protein recovery between 80 and 90% (Bonnerjea et al., 1986). Initial product purity depends on product location (intra- or extracellular). After cell disruption to release intracellular proteins, the product is in a complex solution with other proteins, many with properties similar to the product. For extracellular proteins, most of
### TABLE 3.1: COMMONLY USED RAW MATERIALS IN THE BIOCHEMICAL PROCESS INDUSTRIES (1988 PRICES).

<table>
<thead>
<tr>
<th>RAW MATERIAL</th>
<th>COMMENTS</th>
<th>AVER. USA PRICE ($/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C-Source</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrose monohydrate</td>
<td>91.5% anhydrous dextrose</td>
<td>0.50</td>
</tr>
<tr>
<td>Glucose</td>
<td>Solution 70% w/v</td>
<td>0.32</td>
</tr>
<tr>
<td>Corn syrup</td>
<td>95 Dextrose Equivalent (DE)</td>
<td>0.40</td>
</tr>
<tr>
<td>Corn meal</td>
<td></td>
<td>0.28</td>
</tr>
<tr>
<td>Beet molasses</td>
<td>70% total solids,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50% fermentable sugars</td>
<td>0.10</td>
</tr>
<tr>
<td>Cane molasses</td>
<td>Similar to beet molasses</td>
<td>0.09</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>Refined</td>
<td>0.80</td>
</tr>
<tr>
<td>Corn oil</td>
<td>Refined</td>
<td>0.82</td>
</tr>
<tr>
<td>Ethanol</td>
<td>USP tax free</td>
<td>0.56</td>
</tr>
<tr>
<td>Methanol</td>
<td>Gulf Coast</td>
<td>0.19</td>
</tr>
<tr>
<td>n-alkanes</td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td><strong>N-Source</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonia</td>
<td>Anhydrous, fert. grade</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Aqueous as 29.4%, NH₃ basis</td>
<td>0.21</td>
</tr>
<tr>
<td>Soybean flour</td>
<td>Bagged, 44% protein</td>
<td>0.24</td>
</tr>
<tr>
<td>Cottonseed flour</td>
<td>62% protein</td>
<td>0.51</td>
</tr>
<tr>
<td>Cornsteep liquor</td>
<td>4.5% w/w N</td>
<td>0.15</td>
</tr>
<tr>
<td>Casein</td>
<td>13.5% w/w total N</td>
<td>2.40</td>
</tr>
<tr>
<td>Ammonium Sulfate</td>
<td>Technical</td>
<td>0.12</td>
</tr>
<tr>
<td>Ammonium Nitrate</td>
<td>Fert. grade 33.5% N, bulk</td>
<td>0.14</td>
</tr>
<tr>
<td>Urea</td>
<td>46% N, agricultural grade</td>
<td>0.20</td>
</tr>
<tr>
<td>Yeast</td>
<td>Brewers, debittered</td>
<td>2.40</td>
</tr>
<tr>
<td>Whey</td>
<td>Dried, 4.5% w/w N</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>P, S, Mg, Ca, K, Na-Source</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>USP, granular</td>
<td>1.65</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>Granular, purified</td>
<td>2.20</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td></td>
<td>1.29</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>Agricultural Grade, powder</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Other Raw Materials and Reactor Chemicals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td></td>
<td>285.00</td>
</tr>
<tr>
<td>Penicillin</td>
<td></td>
<td>25.00</td>
</tr>
<tr>
<td>Streptomycin</td>
<td></td>
<td>48.00</td>
</tr>
<tr>
<td>Total animal cell medium</td>
<td>Defined, scale (&gt; 1 m²/day)</td>
<td>1.00</td>
</tr>
<tr>
<td>Antifoam</td>
<td></td>
<td>0.75</td>
</tr>
</tbody>
</table>

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-27-
the contaminants are retained in the cell and are readily removed during biomass removal. Initial product purity also depends on the quality of raw materials used; residual components of complex raw materials, such as molasses, contribute to low initial purity.

<table>
<thead>
<tr>
<th>TYPE OF PRODUCT</th>
<th>YIELD (%)</th>
<th>NUMBER OF STEPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-labile, required in low to medium purity (e.g., SCP, organic solvents)</td>
<td>&gt; 90</td>
<td>2 - 3</td>
</tr>
<tr>
<td>Labile, required in low to high purity (e.g., detergent enzymes, amylases, antibiotics)</td>
<td>60 - 90</td>
<td>3 - 5</td>
</tr>
<tr>
<td>Very labile, required in high purity, extracellular (e.g., monoclonal antibodies)</td>
<td>40 - 60</td>
<td>4 - 6</td>
</tr>
<tr>
<td>Very labile, required in high purity, intracellular (e.g., human growth hormone by recombinant E.coli)</td>
<td>20 - 40</td>
<td>5 - 10</td>
</tr>
</tbody>
</table>

**Final product purity.** High product purity requires a large number of steps and results in low recovery yield.

Based on the above parameters and experience, one can make an estimate of the yield as shown in Table 3.2. After recovery yield estimation, the amount and cost of raw materials required for production can be calculated.

Except for the first, each design level terminates with an economic evaluation that determines whether the project is rejected or whether further work is required to make a final decision on project continuation. We define "Economic Potential" as the difference between the value of product and by-products minus the cost of raw materials divided by the value of product and by-products. This is equivalent to the Gross Margin. At
this design level, the economic potential represents the fraction of revenue not spent for raw materials.

\[
\text{Economic Potential} = \frac{\text{Value of Products and by-Products} - \text{Raw Materials Cost}}{\text{Value of Products and by-Products}}
\]

For a new project, when all production costs are considered and the economic potential is less than 0.3, unless there are opportunities to greatly improve performance or there is a unique strategic return, the project is not economically attractive. Obviously, at this design level, where only the cost of raw materials is taken into account, even projects with higher economic potential should be discarded. The actual threshold value depends on the contribution of raw materials to total manufacturing cost; this can vary from less than 5% (many high value biologicals) to 75% (commodity products) of the total production cost.

This simple analysis provides a quick screening for poor projects. If the product examined is not currently produced in large scale by any method, this level of analysis can be used to check the importance of raw materials cost and also to make a first estimation of the total production cost if some data are available about the contribution of raw materials to total production cost for similar bioproducts (Hacking, 1986).

**LEVEL 2: UPSTREAM SECTION**

At design level 2, the process is decomposed into upstream and downstream sections and design efforts are focused on the upstream
section. The main objective at this level is to synthesize and evaluate the upstream section which consists of raw materials preparation and biosynthesis steps.

At this level, the upstream section is disaggregated into a number of specific unit operations. Figure 3.1 shows a common flowsheet of the upstream section which includes media preparation tanks, very often a continuous sterilizer, seed and production fermentors, a compressor and an air filter to provide sterile air to fermentors, and finally a hold-up tank to isolate the upstream from the downstream section.

During the synthesis of the upstream section, the designer has to decide whether to use a continuous sterilizer or not, has to select the type biocatalyst and the type of bioreactor, has to select the type and estimate the size of the agitation system, the aeration system, the heat transfer system, etc.

But how can this be done? What kind of knowledge is needed and where can it be found?

**BIOCATALYSIS SELECTION**

Selection of type of biocatalyst and bioreactor configuration is the first task. Figure 3.2 shows the options for this task. Selection among the large
Figure 3.1: Common unit operations of upstream section
number of alternatives can be facilitated using domain specific experiential design knowledge.

**Whole cells vs. enzymes.** Enzyme reactors are selected when one or two simple reactions are needed for the conversion of raw materials. Where more complex reaction sequences are required, whole cells are used. Enzymes can be used either in soluble or in immobilized form. Soluble enzymes are preferred (if inexpensive) and there is no need for their removal from the final product (e.g., starch hydrolysis using α-amylase and glucoamylase). There is a trade-off between enzyme cost and immobilization cost.

There are a variety of techniques for immobilizing enzymes. Enzyme reactors can be operated in batch or continuous mode and either as fixed beds, stirred tanks or fluidized beds. Well mixed reactors are advantageous to avoid high substrate concentration and substrate inhibition. Fixed bed or plug flow reactors are suitable for product inhibited reactions. Table 3.3 shows some industrial applications of enzyme and cell reactors.

**Growing vs. nongrowing cells.** Cells can be either growing or nongrowing. Growing cells are used when the product is biomass (e.g., bakers' yeast, single-cell protein) or is intracellular. In other cases, it may be beneficial to use nongrowing or slowly growing cells as biocatalysts. This reduces the amount of raw materials required and facilitates product recovery. Several problems limit the application of nongrowing cells. The formation of some products is growth-associated and growing cells are required. Nongrowing cells may be immobilized thus adding to the cost of support material, creating transfer limitations, and potential contamination problems. Nongrowing and slowly growing cells are used when product synthesis can be uncoupled totally or in part from growth.
FIGURE 3.2: Biocatalysis Decision Tree (adapted and modified from Cooney, 1983)
<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>BIOCATALYST</th>
<th>IMMOBILIZING METHOD</th>
<th>REACTOR TYPE</th>
<th>OPERATING MODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalysed by Enzymes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Amino penicilanic acid</td>
<td>Penicilin acylase</td>
<td>Covalent attachment</td>
<td>Stirred tank</td>
<td>Batch</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Entrapment</td>
<td>Packed bed</td>
<td>Continuous</td>
</tr>
<tr>
<td>HFCS*</td>
<td>Glucose isomerase</td>
<td>Covalent attachment</td>
<td>Packed bed</td>
<td>Continuous</td>
</tr>
<tr>
<td>Glucose</td>
<td>alfa-amylase &amp;</td>
<td>In suspension</td>
<td>Stirred tank</td>
<td>Batch</td>
</tr>
<tr>
<td></td>
<td>glucoamylase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalysed by Bacteria, Fungi, and Molds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>Saccharomyces cerevisiae</td>
<td>Recycle system</td>
<td>Stirred tank</td>
<td>Cont./Batch</td>
</tr>
<tr>
<td>L-lysine</td>
<td>brevibacterium lacofermentum</td>
<td>Entrapped in Ca alginate</td>
<td>Packed bed</td>
<td>Continuous</td>
</tr>
<tr>
<td>Aspartate</td>
<td>Corynebacterium glutamicum</td>
<td>In suspension</td>
<td>Stirred tank</td>
<td>Batch</td>
</tr>
<tr>
<td>Citric acid</td>
<td>Aspergillus niger</td>
<td>In suspension</td>
<td>Stirred tank</td>
<td>Batch</td>
</tr>
<tr>
<td>Xanthan gum</td>
<td>Xanthomonas campestris</td>
<td>In suspension</td>
<td>Stirred tank</td>
<td>Batch</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Penicillium chrysogenum</td>
<td>In suspension</td>
<td>Stirred tank</td>
<td>Batch</td>
</tr>
<tr>
<td>Alkaline Protease</td>
<td>Bacillus subtilis</td>
<td>In suspension</td>
<td>Stirred tank</td>
<td>Batch</td>
</tr>
<tr>
<td>Insulin</td>
<td>Recombinant E.coli</td>
<td>In suspension</td>
<td>Stirred tank</td>
<td>Batch</td>
</tr>
<tr>
<td>Catalysed by Animal and Plant Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monoclonal Antibodies (MAb)</td>
<td></td>
<td>Entrapment</td>
<td>Airlift</td>
<td>Continuous</td>
</tr>
<tr>
<td>Tissue Plasminogen Activator (tPA)</td>
<td></td>
<td>Entrapment</td>
<td>Perfusion</td>
<td>Continuous</td>
</tr>
<tr>
<td>Erythropoietin (EPO)</td>
<td></td>
<td></td>
<td>Fluidized bed</td>
<td>Batch</td>
</tr>
<tr>
<td>Shikonin</td>
<td>Plant cell culture</td>
<td></td>
<td></td>
<td>Batch</td>
</tr>
</tbody>
</table>

* High-fructose corn syrup
This option led to development of several types of immobilized reactors for high cell concentration. The result is lower media consumption and higher productivities. Some examples include: yeast producing ethanol (Oda et al., 1983), animal cells producing monoclonal antibodies, and mixed cultures of bacteria in waste treatment plants (Forster and Wase, 1987).

**Cells in suspension vs. immobilized cells.** Bioreactors using cells in suspension are the workhorse of the biochemical process industry. Figure 3.2 shows the various reactors for immobilized cells. In a CSTR, immobilized cells need to be retained and high shear avoided. Fixed-bed reactors are available in several configurations (e.g. packed-bed, sheet, rotating disc). The rotating disc reactor was developed for mycelial organisms (Blain et al., 1979) which grow on the surface of vertical polypropylene discs rotating half-submerged in the culture medium. In airlift reactors, air is used to circulate reactor contents through an external tube or internal draft tube. These reactors have no rotating parts, are of simple construction and operation, are low power consumers, and have good mass transfer characteristics (Schugerl, 1982). Airlift reactors are used for single-cell protein production.

In recycle systems, cells are separated (e.g. centrifugal separators, membrane devices, settling tanks) and recirculated to the bioreactor. This retains high cell concentration in the bioreactor and results in high productivities. In such systems, cells can be nongrowing or slowly growing. Some ethanol and waste treatment plants operate this way (Hamer, 1988).

**Batch vs. continuous suspension cell reactors.** In continuous reactors, productivity is higher and substrate utilization is more efficient for cell mass, but less efficient for products when compared to batch reactors. The
product concentration is usually lower than in batch reactors, resulting in higher recovery cost. A continuous plant can perform optimally only for the production of one product.

Modifications of batch and continuous reactors are used. When high substrate concentration inhibits growth or product formation, fed-batch operation maintains substrate at an optimal level. Broth can be removed during fermentation in a semibatch approach to continuous operation (Atkinson and Mavituna, 1983). Multiproduct plants operate in batch mode.

The kind of knowledge presented above for biocatalysis selection can be readily documented on the computer in the form of a knowledge base that is used for synthesis.

**EQUIPMENT SIZE AND COST ESTIMATION**

It is possible to do a fairly accurate estimation of capital and operating cost of the upstream section with limited information: 1) *Bioreaction and turnaround time for batch reactors*, 3) *Dilution rate (ratio of flow/volume) for continuous suspension reactors*, 4) *Activity of enzyme catalyst as a function of time and operating conditions*, and 5) *Aeration and agitation requirements of aerobic reactors*, etc.

**Bioreactor Size Estimation.** The bioreactor capacity is estimated by:

\[
\left[ \text{Bioreactor Capacity} \right] = \frac{\left[ \text{Product Output} \right]}{\left[ \text{Bioreactor Productivity} \right] \left[ \text{Recovery Yield} \right]}
\]

Product output and recovery yield were estimated or known from design level 1. Bioreactor productivity is a function of: reactor type, growth rate, and product formation rate.
**Suspension reactors.** Total cycle time (including turnaround time) and dilution rate are respectively required for batch and continuous reactors with suspension cells. Turnaround times in large batch reactors range from 4 to 18 h. Growth and product formation rates depend on the specific microorganism and its growth conditions; they are estimated from a growth model or by analogy to similar processes. Models of growth and product formation and equations for bioreactor size estimation are available in many biochemical engineering textbooks (Wang et al., 1979; Aiba et al., 1973; Bailey and Ollis, 1986).

**Enzyme reactors** are sized as if they were heterogeneous catalytic chemical reactors. The enzyme life and its deactivation as a function of time and operating conditions must be known (Wang et al., 1979).

Design information for other reactor types can be found in: membrane cell reactors (Webster et al., 1979), membrane enzyme reactors (Iorio et al., 1984), air-lift reactors (Siegel et al., 1988), fluidized-bed and trickle-bed reactors (Scott, 1983), rotating disc and drum bioreactors (Anderson et al., 1980), and for many bioreactor types (Atkinson, 1974; Atkinson and Mavituna, 1983).

**Agitator, Compressor, and Heat Transfer System Size Estimation.** The oxygen requirement for aerobic systems as a function of time is known or can be estimated from design level 1 data (Roels, 1983). This variable depends on the carbon source degree of reduction (Wang et al., 1979). This demand along with the mass transfer characteristics of the broth (i.e. viscosity) determine the aeration and agitation requirement.

Aeration rates in stirred bioreactors usually do not exceed 1 VVM (volume of air per volume of broth per minute) to avoid foaming problems. Agitation power ranges from less than 1 to more than 7 kw/m³, depending
on cell concentration, growth rate, product synthesis rate, and broth viscosity (Aiba et al., 1973). Systems of mild agitation, such as fermentation of *E. coli*, require 0.2 to 1 kw/m³, antibiotics production by filamentous microorganisms requires 1.5 to 3.5 kw/m³, yeast production requires 3.5 to 5.0 kw/m³, and xanthan gum production by *Xanthomonas campestris* requires more than 7 kw/m³. For anaerobic bioreaction systems, agitation is required only for mixing. In animal cell stirred reactors effort is made to provide adequate aeration and mixing at low agitation rates to avoid high shear rates which cause cell damage. In air-lift reactors, mixing is carried out by aeration resulting in higher required aeration rates (up to 2 VVM) (Dussap et al., 1982). Aeration and agitation rates determine compressor and agitator size.

All bioreaction systems release heat which can be estimated from the energetics of the conversion reaction using the following equation:

\[ \Delta H_R = \sum_{\text{products}} \Delta H_C - \sum_{\text{reactants}} \Delta H_C \]

where \( \Delta H_R \) is the reaction energy that is released as heat and \( \Delta H_C \) is the heat of combustion of a reactant or product. Heat of combustion for many biochemical compounds can be found in the pertinent literature (Thauer et al., 1977; Battley, 1987; Roels, 1983). The use of the above equation presumes that a overall equation can be used to describe the various reactions taking place in a bioreactor.

For aerobic fermentation, the oxygen uptake rate can be used as an easier way to estimate the heat release (Cooney et al., 1968):

\[ Q_t = 0.12Q_{O_2} \]
where $Q_f$ [Kcal/L/h] is the heat release and $Q_{O_2}$ [mmoles/L/h] is the oxygen uptake rate. A theoretical derivation of the above equation can be found in the literature (Birou et al., 1987). In the design of the heat transfer system, the rate of heat production due to metabolic activity and due to stirring must be balanced by the heat loss resulting from evaporation and radiation plus heat removal by the cooling system (the jacket of the bioreactor and cooling coils).

Mass and heat transfer limitations are the most common bottlenecks in scaling-up bioreaction systems.

**Sterilizer Size Estimation.** Continuous heat sterilization results in lower decomposition of nutrients, requires less steam, and reduces significantly the turnaround time (by 3 to 5 h) of batch fermentors. Presently, there is a tendency to use continuous heat sterilization whenever the production fermentor is larger than 10 m$^3$ or whenever the production fermentor is utilized more than 85% of the time. Continuous sterilizers are usually sized to fill a production fermentor with sterilized media in 3-5 h. Animal cell culture media are usually sterilized using membrane microfiltration units to avoid heat destruction of nutrients.

**Media Storage-Preparation Tanks and Seed Fermentors Size Estimation.** The size of media storage tanks depends on several parameters: type of raw materials, storage stability, seasonal supply, transportation (e.g., by truck, railroad, marine transport). The capacity of storage tanks may range from days to months of supply. Media preparation tank size depends on whether or not a continuous sterilizer is used. If the production fermentor is used as batch sterilizer then smaller tanks are needed because some media preparation mixing can take place in the
fermentor. The size of seed fermentors is calculated as a fraction (usually 5 - 10 %) of the production fermentors.

After the equipment size estimation one proceeds with the estimation of the capital and production cost items of the upstream section. Bioreactors and compressors are the most expensive pieces of equipment in the upstream section. Some useful economic data can be found in the pertinent literature (Kalk and Langlykke, 1985; Reisman, 1988). Electricity consumed by compressors, fermentor agitators, and water chillers is usually a significant part of the production cost of the upstream section. The economic potential at this level is defined as:

\[
\frac{\text{Economic Potential}}{\text{Value of Product and by-Products}} = \frac{\text{Value of Product and by-Products}}{\text{Raw Materials Cost}} \times \frac{\text{Raw Materials Cost}}{\text{Annualized Cost of Upstream Section}} \times \frac{\text{Annualized Cost of Upstream Section}}{\text{Value of Product and by-Products}}
\]

All projects with an economic potential of less than 0.3 are discarded as unattractive. Project assessment at this level is tighter because the cost of the upstream section (including raw materials) can be as high as 85% of the production cost. This cost is high for low value biochemical products. However, this cost is a strong function of recovery yield. The lower the recovery yield, the larger the equipment size in the upstream section and the greater the demand for raw materials. One can reduce upstream section cost by improving downstream section performance. This underscores the interaction between the two sections and the need for a global view in biochemical plant design. At this design level, economic criteria that make use of the capital investment (e.g., Return On Investment, Pay Back Time, Internal Rate of Return, etc.) can be applied.
LEVEL 3: DOWNSTREAM SECTION

At design level 3, the effort focuses on the downstream section. At this level, the designer has to do significant synthetic work in sequencing unit operations that will carry out product recovery and purification to meet specifications. It is necessary to estimate the operating conditions of the unit operations that are as close to the optimum as possible. This is a complex problem because the number of process steps varies from 2 to more than 10 and for each process step there are alternative unit operations. Because of the complexity involved in synthesizing the downstream section, a systematic heuristic procedure to handle this problem has been developed (see next section).

For a sequence of unit operations, material and energy balances are formulated, the equipment sized and the capital cost estimated. Finally, the operating costs (labor, depreciation, process chemicals, utilities, etc.) are calculated. The design effort terminates at this level with the calculation of the economic potential, which is defined as:

\[
\frac{\text{Economic Potential}}{\text{Value of Product and by-Products}} = \frac{\text{Value of Product}}{\text{Cost}} \cdot \left[\frac{\text{Annualized Cost of Upstream Section}}{\text{Raw Materials}}\right] \cdot \left[\frac{\text{Annualized Cost of Downstream Section}}{\text{Value of Product and by-Products}}\right]
\]

This is the complete economic potential because at this level all the major manufacturing costs are considered. If the project still seems
attractive with an economic potential > 0.3, it has a fairly high chance of success. Pilot plant verification of the critical assumptions usually follows to justify continuation of the project.

3.3 A Heuristic Approach to the Synthesis of Downstream Processing of Biological Materials

Synthesis of downstream biochemical flowsheets is a difficult task. The lack of predictive models for most unit operations and the large number of alternatives renders rigorous solution of the problem a mathematical conundrum. This was realized several years ago for the synthesis of chemical processes (Nishida et al., 1981) and led to development of other synthetic approaches (heuristic, hybrid, etc.). The heuristic approach tries to limit the solution space by using rules developed through previous design and operation experience. Some of those heuristics are general and even hold for the synthesis of biochemical separation processes. Four such heuristics are:

1) **Remove the most plentiful component first.**
2) **Remove the easiest to remove first.**
3) **Make the most difficult and most expensive separations last.**
4) **Select processes that make use of the greatest differences in the properties of the product and its impurities.**

A heuristic approach to downstream biochemical flowsheets synthesis is adopted here. First, a block diagram of the main process steps of the
downstream section is developed and second, the alternative unit operations for each step are selected. Development of a block diagram is based on the generalized structure of Figure 3.3. For each product category (intracellular or extracellular) several branches exist in the main pathway. Selection among the branches and alternative unit operations is based on the properties of the product, the properties of the impurities, and the properties of the producing microorganism.

The structure of the generalized block diagram for downstream sections shown in Figure 3.3 can be followed in the development of a knowledge based system that carries out synthesis of downstream sections. Production rules can be organized into groups (knowledge islands) of related information according to various process steps depicted on Figure 3.3. There can be a group of rules, for example, that selects an alternative unit operation for cell harvesting, another group for cell disruption, etc.

3.3.1 PRIMARY RECOVERY STAGES

Primary recovery comprises the first steps of downstream processing where some purification and broth volume reduction occurs. The first step to develop a block diagram for a new process is to distinguish between intracellular and extracellular products (Figure 3.3). Almost all low molecular weight and many high molecular weight bioproducts are extracellular products. Their recovery and purification is easier compared to intracellular bioproducts due to lower amount of impurities present.

Intracellular products remain inside the cell; in the case of proteins, they may be either native or denatured; the denatured intracellular products often form insoluble inclusion bodies. Most recombinant
Figure 3.3: Contemporary Downstream Processing of Biological Materials
eukaryotic proteins produced by prokaryotic microorganisms remain inside the host cell and constitute intracellular products. Some intracellular products remain in the periplasmic space between the cell membrane and the cell wall.

3.3.1.1 INTRACELLULAR PRODUCTS

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

As seen in Figure 3.3, centrifugation and membrane filtration (both micro- and ultrafiltration) are the only techniques used for large-scale cell harvesting. Centrifugation has advantages for large and dense microorganisms (diameter > 2 μm and density > 1030 kg/m³). For instance, centrifugation is very efficient for harvesting yeast. For smaller microorganisms, various coagulation techniques can be used to increase the size of the settling particles. Membrane filtration has advantages for harvesting small cells. If the filtrate flux on *E. coli* (or similar size microorganisms) broth is greater than 20 L/m²-h, membrane filtration becomes cost competitive (Petrides, 1987). Another advantage of membrane filtration is in product recovery. Centrifuge cell loss is 1 to 5%. With membrane filters, all cells are recovered unless there is disruption or lysis or ripped membranes.

Disc-stack centrifuges are most commonly used for cell harvesting. Decenter centrifuges find significant applications, especially when successful coagulation techniques are applied (Brunner and Hemfort, 1988; Axelsson, 1985). With regard to membrane filtration systems, micro- and
ultrafiltration units are used and several membrane geometries are available: hollow fiber, flat sheet, spiral, etc. (McGregor, 1986).

**Cell Disruption.** Cell disruption is usually the second step for intracellular products. It is used to release product from the cell. Disruption of bacteria and yeast is carried out either by high pressure homogenizers or bead mills (Kula and Schutte, 1987; White and Marcus, 1988; Engler, 1985). For high capacities (several m³/h) only high pressure homogenizers are practical. Osmotic shock may be used for periplasmic protein release.

Before disruption the concentrate is diluted (5-10%) with a "lysis buffer" to create conditions to minimize product degradation upon release from the cell. For hard to disrupt microorganisms, multiple homogenizer passes at 500-1000 bar are required. If the product forms inclusion bodies additional passes are required, first, to release the inclusion bodies and second, to create very small cell debris particles to facilitate separation of inclusion bodies from cell debris in the next step.

Some product protein degradation occurs during cell disruption due to high shear and oxidation (Charm and Wong, 1981; Virkar et al., 1981). Generated heat contributes to product degradation. In high pressure homogenizers nearly 99% of the pumping energy dissipates to heat with a temperature rise of about 2.2 °C per 100 bar.

**Removal of Cell Debris.** After cell disruption and the release of product protein, debris is removed by single or combinations of a variety of unit operations: centrifugation, microfiltration, rotary vacuum filtration, press filtration, depth filtration, extraction.
**Soluble product.** When the product is soluble, it is recovered during cell debris removal either in the light phase of a centrifuge or in the permeate of a filter. Centrifuges efficiently separate only fairly large particles of cell debris (greater than 0.5 μm of Stokes's diameter). Therefore, when a centrifuge is used for cell debris removal, a polishing filtration step must follow to remove small debris particles which might otherwise cause severe problems in processes downstream such as chromatography. Various polishing filters can be used (e.g., depth filters, press filters, candle filters, rotary vacuum filters, microfilters, etc.). These filters also can be used for cell debris removal without a centrifuge. It is very difficult to predict a priori which filter performs best for a specific product. When microfilters are used for cell debris removal, some degree of diafiltration is required; the transmission coefficient of the product through the membrane of the microfilter is always less than unity (Datar, 1985). With microfilters, the solution can be concentrated only up to about 40-50% v/v of total solids. Therefore, if one did only a single microfiltration step, a significant amount of product would remain in the concentrate stream and be lost. To reduce product loss, the concentrate stream is diluted and diafiltered. If the plant operates continuously, diafiltration results in multiple microfiltration units in series. If the plant or this process step operates in a batch mode, water is added continuously to the feed tank until the product concentration in the feed tank drops below a certain level.

**Insoluble product.** When the product is insoluble (e.g., inclusion bodies) it must be separated from other particles, then dissolved and refolded. This is a common problem for many eukaryotic proteins produced by prokaryotic microorganisms (for instance, human growth hormone produced by *E.coli*). The foreign proteins form inclusion bodies inside the
host cell. Fortunately, the inclusion bodies are almost pure protein (greater than 75%) and most of the time are of a large average diameter (0.3 - 1.0 μm) and high density (1.3 - 1.5 g/cm³) (Taylor et al., 1986); inclusion bodies can be separated from cell debris with a disc-stack centrifuge. Further purification is done by resuspension and recentrifugation of the heavy phase. After 2-3 washings, the inclusion body suspension is clean. However, instead of simply diluting with pure water, a detergent solution and/or low concentration of a chaotropic agent can be used to facilitate the removal of other contaminants. The pH and the ionic strength of the solution are adjusted to reduce the hydrophobicity of the cell debris particles.

**Extraction for Cell Debris Removal.** Product separation from debris can also be carried out by extraction. Organic solvents and polymer solutions are commonly used extractants. The criteria for extractant selection are: the partition coefficient of the product should be higher than the contaminants; the extractant should not degrade the product; it should not be expensive; and it should be easy to recover or dispose.

Aqueous two-phase systems have found a few applications for recovery of proteins. A procedure for dehydrogenase extraction is described by Kroner et al., (1982), and the simultaneous isolation of two enzymes, 1,4-α-glucan phosphorylase and pullulanase, by Hustedt et al., (1978). Either two different polymers (e.g., PEG and Dextran) or one polymer and one salt (e.g., K₃PO₄ or Na₂SO₄) are used.

Product separation from debris and simultaneous concentration can be achieved by adsorptive extraction techniques (Palmer, 1977). Ion exchange and affinity adsorbents are the most commonly used. The
disrupted cells and product are mixed in a stirred tank with an adsorbent. A washing step follows product adsorption where most of the cell debris and contaminants are washed out. These techniques can be modified to operate continuously (Pungor et al., 1987). In this instance, adsorption occurs continuously in one tank, the product-depleted broth is filtered, and finally the product is eluted continuously in a second tank and the adsorbent beads are recirculated. An advantage of extraction compared to new biochemical unit operations is its high scaleability.

3.3.1.2 EXTRACELLULAR PRODUCTS

**Biomass Removal.** Biomass removal is usually the first step of downstream processing of extracellular products. This step is in agreement with the second generic heuristic, "Remove the easiest to remove first". It can be accomplished by several unit operations: disc-stack or decanter centrifugation, rotary vacuum filtration, press filtration, microfiltration, ultrafiltration, and flotation. Each has advantages and disadvantages for different products and microorganisms making the selection problem difficult.

**Rotary vacuum filtration**, especially with precoat, is widely used for removal of mycelial organisms (Dlouhy and Dahlstrom, 1968). It can operate continuously for long periods of time. The filtrate flux is usually higher than 200 L/m²-h and may reach 1000 L/m²-h. The most important disadvantage of this unit is the problem with the disposal of the mixture of filter-aid and biomass. Stringent environmental laws have made it costly to dispose of such solid materials. Filter-aid is added in equal or higher amounts than biomass. As a conclusion, in the area where a new plant is
going to be built, if disposal of filter aid is allowed at a low cost then a rotary vacuum filter is still a very good choice. Otherwise, look for one of the next alternatives.

**Centrifugation.** Disc-stack and decanter centrifuges are used at the large scale (Brunner and Hemfort, 1988; Axelsson, 1985). Of the two, disc-stack centrifuges operate at higher rotational speeds and remove smaller and lighter microorganisms. With the use of coagulating agents, the decanter centrifuge performance improves and the solution to the problem of selecting between the two types becomes less obvious. Centrifugation does not require filter aid and this is a significant advantage compared to rotary vacuum filtration. The centrifuge paste contains 30-50% v/v extracellular liquid and product dissolved in that liquid is lost if the paste is not washed and recentrifuged.

**Press filtration.** Press filters have limited application for biomass removal. Recently, they have been significantly improved by automation and appear promising for some polishing operations (Zievers, 1988). These filters also require filter aid and have the resultant disposal problem.

**Membrane filtration.** With membrane filters (micro/ultrafilters) the extracellular product passes through the membrane while biomass and other insolubles remain in the concentrate. To recover product remaining in the concentrate, diafiltration is used. Membrane filters are used for biomass removal mainly in recovery of low molecular weight products (e.g., antibiotics from mycelia, Gravatt and Molnar, 1986). For high molecular weight products, the problems of product rejection and denaturation occur. Membrane filtration is a rapidly evolving technology and the applications are increasing significantly.
3.3.2 HIGH RESOLUTION STAGES

After primary product separation, purification is completed in the high resolution stages. If the product is soluble, product concentration is usually the first step. If the product is denatured and insoluble, first it is renatured and then purified.

**Concentration of Product Solution.** After primary separation, the product is in a dilute solution. It is desirable to reduce the amount of material by concentration. The most commonly used alternatives are: ultrafiltration, evaporation, reverse osmosis, adsorption, precipitation, extraction and distillation.

*Ultrafiltration* is used extensively for protein concentration (Sirkar and Prasad, 1986). The molecular weight cut-off of the membrane is selected to retain the product while allowing undesired impurities to pass through the membrane. Purification during concentration and low operating temperatures are advantages of ultrafiltration over evaporation. The typical operating pressure is 2-5 bar and the average flux is 20-50 L/m²-h (Beaton, 1977; Le and Howell, 1985).

*Reverse Osmosis* units have smaller membrane pore sizes and are used for the concentration of medium to low molecular weight product solutions (Kalyanpur et al.). They operate at high pressures (40-60 bar) and low temperature (Coogle, 1985).

*Evaporation.* Thin-film rotating evaporators can operate at low temperatures (40-50 °C) under vacuum (Alfa-Laval, #1). These units compete in the market with ultrafiltration and reverse osmosis for concentration of both low and high molecular weight compounds. One
disadvantage of evaporators compared to ultrafilters is the lack of any purification during concentration. One important advantage is their ability to concentrate to higher total solids concentration.

Precipitation is used for concentration and purification (Bell et al., 1983). Salts, solvents and polymers are commonly used to selectively precipitate compounds of interest (Chan et al., 1986). Precipitation often follows an extraction carried out by a polymer/salt (e.g. PEG and potassium phosphate) system. When the product is recovered in the polymer-rich phase, precipitation is accomplished by addition of more polymer. It is important for economic reasons to recycle materials. Precipitation is used to remove contaminants, i.e. nucleic acids, by adding MnSO₄ or streptomycin sulfate.

Distillation is used for organic solvents recovery (e.g. ethanol, acetic acid, etc.) (Moon, 1985).

Renaturation. Eukaryotic proteins produced by prokaryotic microorganisms often form insoluble inclusion bodies in the host cell (King, 1986). These inclusion bodies can be solubilized by 6M guanidine hydrochloride or other chaotropic salt solutions with a reducing agent, such as 0.5M 2-mercaptoethanol or dithiotrietol solution, to reduce disulfide bonds (Fish et al., 1985; Holzman et al., 1986; Marston, 1986; Yano and Irie, 1975).

After denatured protein solubilization, guanidine hydrochloride is exchanged with buffer using chromatography or diafiltration. The protein solution is diluted to about 0.05% w/v in 4.5M urea solution. Dilution is necessary to minimize intermolecular interactions which occur during product reoxidation and can lead to product inactivation. The chemicals
and conditions used for the renaturation of denatured proteins vary from product to product. The ones described above are typical for porcine somatotropin (pGH).

**Final Purification.** The final purification steps are dependent on the final required purity. Pharmaceutical products require high purity while industrial products require lower purity. For products of relatively low purity (e.g., detergent enzymes), the final purification step is dehydration or more generally a solvent removal step. For high purity products, final purification steps usually involve a combination of chromatographic and filtration units (Bjurstrom, 1985).

**Dehydration or Solvent Removal** is achieved with dryers. Spray dryers (Masters, 1985; Neubeck, 1980) and fluid bed dryers (Wentz and Thygeson, 1988) are used when products can withstand temperatures of 50-100 °C. Freeze dryers are used for products that degrade at high temperatures (Snowman, 1988). Freeze dryers have high capital and operating costs and should be avoided if possible.

**Chromatography** is typically done later in a process in agreement with the third generic heuristic "Make the most difficult and most expensive separations last". With the previous separation steps, a large fraction of contaminants are removed reducing the amount of material to be treated. A 50-100 fold volumetric reduction is common for high value biological products resulting in a protein content of 5-7 % w/v of the feed stream to chromatographic units (Pharmacia, #1).
Chromatography units include: ion exchange, gel filtration, affinity, hydrophobic interaction, chromatophocusing. A sequence of units is usually required to achieve the desired product purity and the fourth generic heuristic -- "Select processes that make use of the greatest differences in the properties of the product and its contaminants" -- is a good guide for selecting and sequencing these units (Wheelwright, 1987).

**Gel filtration** is based on molecular size and has two main applications: removal of small molecules from proteins (desalting or group separation) and removal of contaminant proteins from product protein. Gel filtration competes with ultrafiltration for desalting. For protein fractionation, gel filtration is slow compared to other chromatographic techniques. It is most suitable as a final purification to remove contaminants from small product volume. Purification by gel filtration usually results in dilution requiring concentration further downstream. A detailed description of process scale gel filtration with design data can be found in the pertinent literature (Janson and Hedman, 1982; Delaney, 1980).

The first large scale applications of gel filtration were in the group separations such as removal of sugars and salts from whey protein solution (Lindquist and Williams, 1973) and removal of ethanol from albumin (Friedli and Kistler, 1980), etc. The first large scale application of gel filtration for protein fractionation was in the purification of insulin (Janson, 1971). The fractionation of human serum proteins followed (Curling, 1980).

**Ion exchange** separation is based on molecular charge. Most biomolecules are polar and charged; since charge depends on pH and ionic strength, either anionin or cationin resins can be used. In practice,
however, the choice is usually determined by the pH stability of the product that is purified.

Ion exchange is important for purification because it provides high binding capacity (50 g of protein per liter of ion exchanger is common), a large variety of elution conditions (e.g., change in pH and ionic strength with continuous and stepwise gradients), and availability of relatively inexpensive resins. Ion exchange is suitable at nearly any stage of product purification. It can handle large quantities of protein solution with significant purification and concentration (Curling et al., 1984; Pharmacia, #1). Ion exchange chromatography requires shorter columns (average of 25 cm) compared to gel filtration. Scale up is achieved by increasing column diameter and not bed height. Productivity depends on total cycle time which in turn depends on linear flow rates which vary from hundreds to thousands L/m²-h, depending on adsorbent and applied flow pressure.

Some applications of ion exchange include: amino acid recovery and purification (e.g. L-lysine, L-tryptophan), plasma fractionation (Bjorling, 1972), insulin purification (Pharmacia, #1), lysozyme purification (Notarianni and Ghielmetti, 1971), and antibiotics purification (Belter, 1985).

**Affinity Chromatography.** Separation by affinity or molecular recognition is based on specific interactions between biomolecules (Hill and Hirtenstein, 1988; Kopperschlager et al., 1982). A detailed description of affinity chromatography theory and large-scale design can be found in (Yang and Tsao, 1982a,b; Janson and Hedman, 1982).

Affinity chromatography is appropriate at any stage of product purification and is particularly useful for handling large volumes of material with low product concentration and many contaminants
(Pharmacia, #1). Its high specificity results in substantial purification (up to several thousand fold of purification factor) and high recovery yields (usually > 90%). The high cost of ligand immobilization limit the application of affinity chromatography to high value products, such as interferon (Knight and Fahey, 1981; Staehelin et al., 1981) and antithrombin III (Nishikawa, 1978).

**Hydrophobic Interaction Chromatography** (HIC) is based on nonpolar (hydrophobic) interactions (Er-El, 1985). Many biological substances have nonpolar regions that interact with adsorbent-containing hydrophobic groups; immobilized phenyl and octyl groups have frequently been used for this purpose. HIC can be utilized at various stages of purification but is particularly effective when the product is in the presence of high salt concentration, for instance after product precipitation by salt; this lowers the protein solubility and increases its tendency to interact with nonpolar surfaces (Curling, 1984). It is suitable between two ion exchange steps for buffer exchange (this is in agreement with the fourth generic heuristic). Elution of adsorbed proteins is achieved by decreasing ionic strength or/and lowering polarity, such as by adding ethylene glycol (Chase, 1988).

**Chromatofocusing** is based on the isoelectric point of a molecule in a pH gradient rather than on the overall charge (Pharmacia, #2; Sluyterman and Elgersma, 1978). The gradient is created by titrating the gel with a specially prepared buffer. Chromatofocusing offers very high resolution and the gels have high capacities for proteins and can tolerate very high flowrates, which makes them very suitable for large-scale operation (Curling et al., 1984). Chromatofocusing is suitable for the final stages of purification. A problem is the high cost of ampholites used in this process.
**High Performance Liquid Chromatography (HPLC)** is not a different type of chromatography but rather a means for improving the speed of chromatographic techniques (Lottspeich et al., 1981). HPLC methods are available for existing types of chromatography and find increasing applications in purification of small e.g. antibiotics, amino acids (Dwyer et al., 1984) and large molecules (Scott, 1984).

**Finishing Operations**

*Product Sterilization.* Membrane microfilters (0.2 - 0.45 μm pore size) are commonly used to sterilize biopharmaceutical solutions by removing any bacterial contamination. A limitation in microfiltration is its inability to remove pyrogens and viruses; these must be removed at earlier stages (Weary and Pearson III, 1988). To further minimize the likelihood of pyrogen contamination, water used in purification is pyrogen free.

*Product Stabilization.* To increase product shelf-life, often stabilizing agents are used: salts (e.g., ammonium sulfate, calcium chloride, sodium chloride) and polyols (glycerol, propylene glycol). Some products are stable only as solids. The most common dehydration technique for labile products is freeze-drying (Snowman, 1988; Moore, 1983).
Chapter 4

Implementation Features

4.1 Architectural Overview

BioDesigner is composed of three basic components: user interface, synthetic component, and analytic component (see Figure 4.1). The interface and the analytic component are written in THINK C. The analytic component carries out the material and energy balances, estimates the size and cost of equipment, carries out the economic evaluation, handles the storage of information on the hard disk, and implements the facilities of the first level of design. C was the programming language of choice mainly because it supports advanced data structures and runtime memory allocation. Advanced data structures are needed to store and efficiently manipulate information about various objects, such as unit operations, chemical components, material streams, etc. The runtime memory allocation feature allows the program to handle flowsheets of any number of unit operations, streams, etc. (the actual computer memory is the only limitation).

The synthetic component consists of two Knowledge Bases and the inference engine of Nexpert Object. The first knowledge base contains information for synthesizing upstream sections whereas the second contains information for synthesizing downstream sections. Nexpert Object is a commercial expert system shell marketed by Neuron Data, Inc. Nexpert Object was selected among several expert shells because: 1) it
Figure 4.1: BioDesigner's System Architecture
provides an object-oriented environment that facilitates representation of declarative knowledge; 2) it can be fairly easily interfaced to programs that are written in the C programming language; and 3) its development version has a user friendly interface that facilitates development of knowledge bases. Nexpert Object is used in its dynamic library version (NDL) and is called from within BioDesigner as an external dynamic subroutine (see Figure 4.2). The interfacing is accomplished through the Application Program Interface (API) library provided by Neuron Data. NDL is loaded into computer memory only when the synthetic capabilities of the program are used. This architecture allows the analytic component of BioDesigner to be used, if desired, as an independent application with lower memory requirements (less than 1 Mega Byte).

4.2 Object Representation

BioDesigner represents and stores information of several types of objects: unit operations, streams, chemical components, flowsheets, etc. Even though the analytic component of BioDesigner is written in plain C and not object oriented, several representational features of object-oriented programming are used. For instance, a hierarchy of user defined C data structures (similar to class hierarchies in object-oriented environments) is used to store information about unit operations. Memory for these structures is allocated dynamically at runtime. Hence, a flowsheet can have any number of components, unit operations, streams, etc.

4.2.1 Unit Operation C Structures
Two C structures are used to represent information about a unit operation (see Figure 4.3). The first one is the same for all unit operations because it stores common information, such as the name of a unit, the purpose, the picture ID ... the power requirement, the heating or cooling duty, etc. The second structure is specific to each unit operation because it stores unit specific information. The information in the second data structure is accessed through the theModel pointer. The second C data structure in Figure 4.3 is for the simplest unit operation, a centrifugal pump. The only information stored for a centrifugal pump includes: the pressure change, the material of construction, and the pumping efficiency.

Figure 4.4 shows the equivalent data structure representation of unit operations in an object-oriented programming environment. The 'UnitOper' class corresponds to the common C structure. The various subclasses correspond to unit specific C structures. Finally, the triangles represent instances of unit operations.

The various unit operation objects of a flowsheet form a linked list (see Figure 4.6). Having a pointer to the head of the list, one can access the information of any unit operation object.

4.2.2 Component and Stream C Structures

Figure 4.5 shows the component and stream C structures. The Component object has the properties: Name, MW (Molecular Weight), PartSize (Particle Size), and Density. The Stream object has the properties, From_Unit (name of source unit operation), To_Unit (name of destination unit operation), TotalMassFlow, Temperature, Pressure, etc.
To store the flowrate of each component in a stream (CompMassFlow), the **St_Comp** structure is used, which also stores information about the location of each component (Intracellular or Extracellular). For each stream object, several such St_Comp objects (equal to the number of Components used in the flowsheet) form a list, which is pointed by the 'StCompList' pointer.

### 4.2.3 Flowsheet C Structure

Figure 4.6 shows the flowsheet data structure. The flowsheet object has the properties, *Name* (name of a flowsheet) and *Description* (description of a flowsheet). It also stores pointers to the heads of the unit operations linked list, the streams linked list, and the components linked list. This data structure facilitates representation and manipulation of multiple flowsheets in the computer memory. Having a pointer to a flowsheet object, one can access all information associated with that flowsheet.

### 4.3 Flowsheet Connectivity

The topology and connectivity of a flowsheet is represented through the *UnitOp* and *Stream* objects. A stream object stores the names of the source (From_Unit) and destination (To_Unit) unit operations (Figure 4.5). A stream that has no source unit operation is called 'Feed' stream whereas a stream that has no destination unit operation is called 'Product' stream (read User's Guide in Appendix I for more details). Feed and Product streams are distinguished by their unique symbols that are drawn
automatically by BioDesigner. Similarly, a UnitOp object stores information about its associated streams. During the analysis of a design case, the connectivity of a flowsheet is established by using the above information.

4.4 The Intelligent User Interface

The design and implementation of BioDesigner's user interface was intended to create a system that is simple and easy to learn. The interface is fully graphical and eliminates the need for development of the FORTRAN-like input files that most mainframe process simulators require. The guidelines set by Apple Computer for interface development were closely followed (Apple Computer, 1987). The development of BioDesigner was done in an Outside-In and Top-Down manner (Simpson, 1986). The potential user and the outputs of the program were given foremost consideration. BioDesigner's event driven, intelligent interface improves significantly the communication between the user and the computer and reduces the learning period. Some features and implementation considerations of BioDesigner's interface development include:

User definition. The average user of BioDesigner is expected to be a chemical/biochemical engineer or a student in Chemical Engineering who knows the basics of how to use the Macintosh computer. He/she is expected to have some familiarity with process flowsheets, material and energy balances, and project economic evaluation. No programming experience is assumed. The user is expected to become an expert user of BioDesigner in hours and not weeks or months as with older systems.
Work and memory minimization. Effort was made to minimize time and work required to carry out a task. The extensive use of advanced graphics has reduced the time to develop a flowsheet to a few minutes. The use of good default values for unit operation variables, economic variables, etc. reduces the initialization time. Effort was made to minimize demands on recall memory through the use of pull-down menus, windowing capabilities, palette, etc. The functionalities of BioDesigner's interface are consistent with most established Macintosh software packages. A characteristic example is the various cursors. The "eraser" cursor is used to erase a unit operation or a stream, the "hand" cursor is used to move a unit operation of a stream's name, etc.

Program forgiveness. Effort was made to plant the seeds of program forgiveness. The interface facilitates experimentation by providing an "Undo" option and allowing the user to save a design case at any stage of development. In the future, no combination of user actions will result in aborting the application. Alert windows are used extensively to warn the user for inadvertent actions. The use of the "Undo" facility will be extended to most functionalities of BioDesigner.

Internal documentation. BioDesigner provides an advanced "Help" facility that minimizes the need for written manuals. The user has quick access to information on "How to Use BioDesigner", information on the various unit operations, cell composition of various microorganisms, media preparation, raw material prices, etc. Several items of the help facility are editable and the user can document his/her own information.

More information on the user interface can be found in the "User's Guide" (Appendix I).
Figure 4.2: BioDesigner - Nexpert Dynamic Library (NDL) Interface
Figure 4.3: Unit Operation C Structures
Figure 4.4: Equivalent Class-Object Representation
struct Stream

char Name[16];
Boolean Initialized;
char From_Unit[16];
char To_Unit[16];
...
....
St_CompPtr StCompList;
double MassFlow;
double Temp;
double Activity;
...
....
struct Stream *next;

Figure 4.5: Stream and Component C Structures
Figure 4.6: Flowsheet C Structure
Chapter 5


5.1 Introduction

As mentioned previously, process synthesis is the selection and arrangement of a set of unit operations capable of producing the desired product at an acceptable cost and quality. Chapter 3 presented general structures of upstream and downstream flowsheet sections. It was mentioned that if one wants to consider all alternative flowsheets, the problem becomes combinatorial and the number of alternatives grows exponentially. It was also mentioned that the number of alternatives can be reduced significantly using domain-specific experiential design knowledge. Experiential knowledge in the form of heuristics and rules of thumb is knowledge accumulated and refined by experts over several years of problem-solving experience in a specific domain. This chapter presents information on how to capture and document this type of knowledge on the computer in an active and easily accessible and modifiable way. Designing a computer program to do this activity is the study of Knowledge Based Expert Systems (KBES), an important field of applied artificial intelligence. KBES are appropriate for dealing with difficult, ill-structured, problems in complex domains, such as the process synthesis problem, typically not amenable to purely algorithmic solutions.
The rest of this chapter presents general information on KBES as well as specific information on the use of KBES in BioDesigner to carry out synthesis of integrated biochemical processes.

5.2 Knowledge Based Expert Systems

The Knowledge Based System programming methodology offers a convenient way to encode the heuristic knowledge of human experts. This approach is an appropriate model of problem solving when the problems to be solved present some or all of the following characteristics (Davis, 1983; Beltramini, 1988):

1) The problem is complex, that is, there are many independent states in the domain and the variations are large and important, and the responses to it are diverse and based on attention to many factors. KBES are interruptable and can shift focus of attention quickly.

2) A purely algorithmic solution is not appropriate.

3) Domain experts are available and are much better that amateurs.

4) The knowledge to be programmed naturally occurs in rule form. In this case the knowledge organization of a KBES resembles the organization of human knowledge, individual rules are said to correspond to units of human problem-solving knowledge.

5) A program's control is extremely complex.

6) The program is expected to be significantly modified over a long period of time.

One readily recognizes several of the above characteristics in process synthesis. The use of KBES seems appropriate for the synthetic task of the overall design problem. A KBES can effectively handle domain-specific
experiential design knowledge to select and order unit operations capable of producing the desired product. A KBES approach, however, seems impractical in handling the analytic tasks of design. Mathematical models that describe the behavior of unit operation models are more efficiently implemented in standard procedural computer languages. To overcome the difficulties and exploit the advantages of the two approaches, BioDesigner has been implemented as a hybrid tool. Its synthetic component is a KBES that makes use of the Nexpert Object expert system shell. On the other hand, its analytic component is written in C. The two components have been interfaced (see Chapter 4) and the results of synthesis can be directly transferred to the analytic component for analysis and evaluation.

KBES can be efficiently built using either an Expert System Shell or an Artificial Intelligence (AI) computer language. An expert system shell usually provides an inference engine and a user interface to develop knowledge bases. Some expert system shells also provide interfaces to commercial databases, spreadsheets, programming languages, etc. An AI language like Prolog or Lisp provides greater flexibility than an expert system shell but usually requires more work to build a prototype KBES. A description of the architecture and main components of KBES follows.

5.2.1 Architecture of a Knowledge Based Expert Systems

Typically, a KBES includes three major components (Sriram, 1988):

1) **Knowledge Base** is a collection of general facts, rules of thumb and causal models of the behavior of the problem domain. A number of
formalisms have been developed to represent knowledge and the most widely used one is the *production system* model. In this formalism, a rule has a condition or premise part (left hand side, LHS) and an action or conclusion part (right hand side, RHS). Uncertainty in the knowledge is represented by means of confidence factors. Frame-based schemes are often also used to represent more efficiently the physical world of the problem.

1) **Context or Data Store** (data memory or working memory) is a workspace of the constructed solution and serves as a global database of symbols representing facts and assertions about the problem. The data are instances of "objects", which may represent either physical objects or facts related to the domain of application, or conceptual objects such as goals related to the problem solving strategy.

3) **Inference Engine** or **Interpreter** is used to monitor the execution of the rules. The interpreter must determine which rules are relevant to a given data memory configuration or state, and choose the specific one to apply. Usually, this selection or control strategy is called "conflict resolution".

The inference engine can be described as a finite-state machine with a cycle consisting of three action states: match rules, select rules, and execute (fire) rules. This mechanism is referred as the *recognize-act* or *situation-action cycle*. This alternation of selection and execution is an essential element of KBES architecture and is responsible for one of their most fundamental characteristics. By choosing each rule for execution on the basis of the total contents of data memory, a complete reevaluation of the control state of the system is done at every cycle. This is distinctly different from the conventional programming languages approach, in which control flow is typically the decision of the process currently executing, and
commonly dependent on only a small fraction of the total number of state variables. KBES are thus sensitive to any change in the entire environment, and potentially reactive to such changes within the scope of a single execution cycle.

The recognition phase of the recognize-act cycle involves selecting a rule for execution, and thus can be further subdivided into selection and conflict resolution. In the selection process, one or more potentially applicable rules is chosen from the set, and passed to the conflict resolution algorithm, which in general chooses one of them. Sensitivity and stability of the system's behavior are dependent upon the conflict resolution strategy (Waterman and Hayes-Roth, 1978). Some of the most common conflict resolution strategies include:

1. Rule order. There is a complete ordering of all rules in the system, and the rules in the conflict set with the highest priority are chosen.

2. Data order. Elements of data memory are ordered, and the rule that matches the elements in the data memory with the highest priority is chosen.

3. Generality order. The most specific rule is chosen.

4. Rule precedence. A precedence network determines the hierarchy.

5. Recency order. Chooses either the most recently executed rule, or the rule containing the most recently matched element of data memory.

The data memory of a KBES is a collection of symbols intended to reflect the state of the world as well as facts and assertions about the world. One important characteristic of the data memory is that it is the only storage medium for all state variables of the system. In particular, unlike procedurally oriented languages there is no provision for separate storage of control state information. There is nothing but the single data base and
all the information to be recorded must go there. This is referred as the
unity of data and control store. This store is universally accessible to every
rule in the system, so that anything put there is potentially detectable by any
rule. Because of the relatively independent nature of the rules and the
reduced amount of control information, a KBES specification does not
prematurely determine the partitioning, control, and data structure of the
ultimate solution.

5.2.2 Production Rules

The use of production rules is an effective way to represent a decision-
making type of knowledge and situation -- action related knowledge. A
production rule is made up of IF condition THEN action or IF premise
THEN conclusion statements. The IF part is commonly referred to as the
Left Hand Side (LHS) of the rule and the THEN part as the Right Hand Side
(RHS). The LHS consists of a number of conditions, where each condition
evaluates as either true or false. These conditions are normally connected
by logical AND and logical OR connectives (Nexpert Object only supports
AND connectives). Given a problem situation that satisfies or matches the
IF part, the action or conclusion specified by the THEN part is performed (a
phenomenon known as rule firing). There can be a multiplicity of
statements in both parts or the rule. Also, in some environments [Nexpert
Object, 1988], the THEN part is branched into two parts, a THEN part made
up of a single statement and a DO part with multiple statements. So instead
of having THEN action or THEN conclusion, a THEN configuration and DO
action or conclusion setup is provided.
Upon firing of a rule, several events can take place. For example, a set of queries may be posed to the user, an external procedural program may be called, new information (facts or hypotheses) may be confirmed and thereafter, added to the system's database, or a control strategy may be directed by the triggered evaluation of other rule sets or tests. In this latter possibility, where rules call other rules, one can establish what is known as a rule network or an inference chain: a sequence of steps or rule applications needed to reach a particular conclusion. With such a network, the reasoning process within the rule based system can be effectively traced. For the rule network behaves like a map or topography by depicting the links between the available rules in the knowledge base.

There are two important ways in which rules can be used in a rule based system; one is called forward chaining (data driven) and the other backward chaining (goal driven) (Waterman, 1986). In forward chaining, the IF portion of rules are matched against facts to establish new facts. In backward chaining, the system starts with what it wants to prove and tries to establish the facts it needs to prove it. In Nexpert Object one can have either one or a combination of the two in a symmetrical way. As Rappaport (1988), the main developer of Nexpert Object, puts it:

Because expert reasoning is different from solving abstract puzzles for the most parts, rules must contain a high level of control information. This allows them to be used in a symmetric fashion. This symmetry avoids having to define the rule artificially as a forward or backward structure. The compiled nature of the knowledge makes it much more suitable for an event-driven, opportunistic general control as opposed to a specific, often unadapted algorithm.

5.2.3 Object-Oriented Representation (Frames)
The expressive power of production rules is poor for defining terms and describing domain objects and static relationships among them. The use of "frames" or "object-oriented representation" is more appropriate for these tasks (Minsky, 1975). Frames provide a structured representation of objects or classes of objects. Frames are ideal for portraying knowledge concepts that can have many features attributed to them, each with a situational value. Using frames, classes of objects can be organized into taxonomies and in this way each class can be described as a specialization (subclass) of other more generic classes. For example, the class separators can be described as specialization of the class equipment, and in turn the class filters can be described as separators plus a set of properties that distinguish filtration from other types of separation methods. The taxonomic relationships among frames enable descriptive information to be shared among multiple frames via inheritance. A frame system also allows the attachment of behavioral information in the form of procedures written in the underlying base language, C, or Pascal in the case of Nexpert Object. These procedures may act as "methods" or "demons". "Methods" associated with classes respond to specific messages providing an object-oriented programming environment. "Demons" are procedures that are automatically activated during access (put or get) to slot values or in a when needed fashion. Methods and demons are inherited by subclasses.

The inheritance mechanism within a frame-based system provides an efficient means for modeling large and complex systems. Attributes, defaults, and methods defined in higher level classes are passed down to more specialized subclasses. In subclasses, there is the possibility of adding new information or modifying the values of inherited ones. The inference
done by a frame-based system, using the inheritance mechanism based on the taxonomy of classes, plays a major role in the overall reasoning of a knowledge system and in the efficient description of domain knowledge. In this sense, classes of objects have a double appeal, first, because they correspond well with the structure of the domain knowledge to be represented, and second, since they suggest directions for efficient processing (inheritance, defaults) by using simple tree traversal algorithms, being much faster in the computation of these values than general deduction methods, such as logic theorem provers or production rule interpreters. On the other hand, logic theorem provers are capable of expressing a wider spectrum of general knowledge, an example of the typical trade-offs between power and efficiency.

An Artificial Intelligence language or an Expert System shell, when used for process synthesis, must allow creation of frames during execution since the number and identity of frames that represent equipment and other objects are not the same for each design.

5.2.4 Advantages of using a KBES approach to carry out synthesis

1) **Modularity**: KBES offer modularity because rules to be invoked are solely dependent on the contents of the data base, and no rule is ever called directly. Thus, the addition or deletion of a rule does not require the modification of any other rule to provide for a call to it. KBES emphasize the decoupling of control flow from the writing of rules. Each rule is designed to be an independent "chunk" of knowledge with its own statement of
relevance. The system is said to be "data driven" because the state of the environment influences the next rule for execution.

2) Explanation of primitive actions: As a consequence of the separation of knowledge and control, and having the rules in a restricted format, it is relatively easy to explain its behavior, since each rule specifies one well-understood piece of knowledge.

3) Reactivity: KBES can cope with unanticipated situations. Unplanned but useful interactions result from applying knowledge when it is appropriate rather than calling on it in predetermined sequences.

5.3 Synthesis in BioDesigner

The selection and ordering of unit operations in BioDesigner is accomplished by two knowledge bases. The first contains experiential knowledge to carry out synthesis of upstream sections while the second carries out synthesis of downstream sections.

Improvement and modification of knowledge bases in BioDesigner is best done using the development version of Nexpert Object that provides advanced editors and a user friendly interface. In the BioDesigner application, however, knowledge bases are processed using the Nexpert Dynamic Library (NDL). NDL provides all the inferencing capabilities of the development version but lacks the user interface. NDL has been embedded in BioDesigner and is used as an external, dynamically loaded, subroutine (see Chapter 4 for more details). A simple user friendly interface has been developed that allows communication between the user and the synthetic component of BioDesigner.
During a synthesis session, the user goes through a dialog with the system providing information about the properties of the product, the producing microorganism, the contaminants, etc. In the current version of BioDesigner, the result of a synthesis session is a feasible flowsheet. A flowsheet developed by the synthetic component of BioDesigner can be automatically transferred to the analytic component for analysis and evaluation.

5.3.1 Heuristic Knowledge in BioDesigner

Heuristic knowledge in BioDesigner is stored in the form of production rules. Production rules express a wide variety of experiential knowledge, ranging from common sense to rare and uncommon knowledge. The following rule is an example of knowledge that can be readily considered as common sense.

\[
\text{IF the microorganism is aerobic,}
\]

\[
\text{THEN include a compressor in the upstream section.}
\]

The following rule presents information which is fairly widely known among biotechnologists but cannot be considered common sense.

\[
\text{IF the type of microorganism is an animal cell,}
\]

\[
\text{THEN use membrane filtration for media sterilization.}
\]

Production rules in the knowledge bases are organized into groups of related rules (knowledge islands). For instance, the rules of the knowledge base that carries out synthesis of downstream sections follow a structure similar to Figure 3.3. There is a group of rules that select a unit operation for cell harvesting, another group for cell disruption, etc. This organization
Figure 5.1: Properties of the Product object

Figure 5.2: Class hierarchy of unit operations used in the knowledge base that synthesizes upstream sections
allows easy expansion and modification of the knowledge bases. The full set of production rules used in BioDesigner is given in Appendix II.

5.3.2 Object Representation in the Synthetic Component of BioDesigner

Information about unit operations, microorganisms, chemical components, etc., is stored in the form of objects supported by the Object-Oriented environment of Nexpert Object. In Nexpert Object, objects have properties that take values and methods that carry out procedures. Objects may also inherit properties and methods from parent objects. Figure 5.1 shows the properties of the Product object. Figure 5.2 shows the Class hierarchy of the unit operation objects in the knowledge base that carries out synthesis of upstream sections. An analogous class hierarchy, with recovery and separation unit operations, is used in the knowledge base that synthesizes downstream sections.
Chapter 6

Analysis in BioDesigner

6.1 Introduction

For a given flowsheet, the analytic component of BioDesigner carries out material and energy balances, sizing and costing of equipment, and economic evaluation. It provides a user friendly interactive interface to allow the rapid development of flowsheets. The analytic component of BioDesigner can be used as a stand-alone application for process analysis tasks. It also can be used in conjunction with the synthetic component. The flow of information in the current version of BioDesigner is mainly from the synthetic to the analytic component. A flowsheet developed by the synthetic component is transferred to the analytic component for analysis and evaluation.

This chapter describes the major components of the analytic component of BioDesigner.

6.2 Development of a Flowsheet

A flowsheet is a set of unit operations ordered in a certain sequence and linked together with streams. Unit operations are represented on the computer screen with standard graphical pictures that either resemble the actual appearance of a unit or describe its functionality. In developing unit
operation pictures, effort was made to follow current industrial practice whenever possible. BioDesigner provides capabilities to move a unit around on the screen, flip it horizontally, erase it, look at and modify its equipment variables, etc. (see User's Guide in Appendix I for more details). The development of a flowsheet is also described in Appendix I. BioDesigner does not require development of an input file to describe a flowsheet, unlike most conventional process simulators. The graphical representation of a flowsheet effectively describes the topology and connectivity of a flowsheet.

A flowsheet can have any number of unit operations, streams, and chemical components because memory for all those objects is allocated dynamically at runtime.

6.3 Chemical Components

Initialization of chemical components is the next step after flowsheet development. Chemical components are fundamental entities in a process analysis tool. They describe compositions of feed, intermediate, and product streams. It is the purpose of most industrial processes to change the relative amounts of the chemical components when transforming raw materials into products by means of chemical reaction, separation, mixing, etc.

In the current version of BioDesigner, chemical components have three global properties: molecular weight (MW), particle size (in μm), and density (in kg/m³). In addition, the product activity basis (in units/g of pure product) and the water content of microorganisms are also globally available. These properties are taken into account by the various unit
operation models in the estimation of material balances and sizing of equipment. The particle size, for example, is used in sizing of filters and centrifuges. Values of additional properties that are required by some unit operations (e.g., viscosity by a disc-stack centrifuge) are specified during initialization of those unit operations.

The limited number of global properties that BioDesigner requires offers it a greater flexibility in handling any type of chemical components. It can handle conventional components as well as complex materials, such as biomass, cell debris, etc.

6.4 Material Streams

A BioDesigner stream represents the flow of material from one unit operation to the next. A stream object stores information about the flowrate of each component, the location of each component (intracellular or extracellular), the total mass flowrate, its name, and several stream properties (Temperature, Pressure, Product Activity, etc.).

The various unit operation objects have ports for inlet and outlet streams that allow them to be connected with other units to form a flowsheet. The drawing of a stream is easily done using the mouse (see Appendix I for more details). Feed and product streams have special symbols to distinguish them from intermediate streams. During initialization of a design case, the user has to specify all the feed streams.

BioDesigner can create a detailed stream report showing the various stream properties, the flowrates of intracellular components, the flowrates of extracellular components, subtotal flowrates, and total stream mass
flowrate. The user can specify the format of stream report selecting among several alternatives (e.g., kg/h, kg/Cycle, etc.) (see Appendix I for more details).

6.5 Unit Operation Models

A unit operation object is represented on the computer screen with a picture. It has one or more input streams (equal to the number of inlet ports) and one or more outlet streams. For each unit operation there is a model that describes its performance. A BioDesigner model refers to the collection of subroutines used to model the unit operation and, in effect, defines the calculation of outlet stream variables from inlet stream(s) information.

The processing of information by a unit operation model is shown in Figure 6.1. The primary function of a unit operation model is to carry out the material and energy balances around a process step and estimate outlet stream variables given inlet stream variables and engineering specifications. The user provides engineering information during initialization of unit operations through unit specific dialog windows (see Appendix I for more details). For most of the engineering variables there are good default values which can be used during a first pass until better values become available.

Material balances in BioDesigner are estimated in a sequential modular approach. The various unit operations of a flowsheet are sequenced according to their calculation order. Every unit operation model estimates the composition and flowrate of its outlet streams given its inlet
Figure 6.1: Processing of Information by a Unit Operation Object
streams and the unit specific engineering information. The current version of BioDesigner does not estimate stream enthalpy but estimates amount of utilities required by the various unit operations of a flowsheet.

6.6 Equipment Size and Cost Estimation - Economic Evaluation

BioDesigner provides facilities to carry out a complete economic evaluation of a project idea that deals with the production of a biological product. More specifically, it estimates purchase cost of equipment, cost of fixed capital investment, annual operating cost, and lastly carries out profitability and cash flow analyses.

During flowsheet initialization, the user specifies several economic parameters which include: type of depreciation, capital outlay profile, debt fraction and loan terms, project life, capital gain taxes, year of analysis, duration of construction, prices of raw materials and process chemicals, etc. In addition, for most of these variables there are preliminary default values that can be used during a first pass. The use of good default values has proven very successful in reducing flowsheet initialization time. The user also has the option to provide his/her own values for purchase cost of equipment and other economic variables to override the ones that are estimated by the system (see Appendix I for more details). Figure 6.2 shows the flow of information for equipment size and purchase cost estimation and economic evaluation.

BioDesigner is intended to provide cost estimates for preliminary design with accuracy in the range of ±30%. The cost data come from articles or books and from vendor quotations. All costs are factored to a June 1988 basis, or a chemical engineering index number of 341.6.
Figure 6.2: Flow of Information in Sizing, Costing, and Economic Evaluation
Equipment purchase cost is estimated by equations which are a function of equipment capacity, material of construction, and some design characteristics. The fixed capital investment is estimated based on the total purchase cost of equipment using multipliers (see design case example in Chapter 8). Great care has been given to the proper estimation of the cost of off-sites, since they represent a significant fraction of the cost of a new plant. The various items of the annual operating cost (e.g., raw materials, process chemicals, labor, utilities, waste treatment, etc.) are estimated at a detailed level. Profitability and cash flow analysis are the last items of the economic evaluation and show the attractiveness of a project idea from an economic point of view.

Finally, BioDesigner creates a detailed economic evaluation report with formatted tables that can be directly inserted into a project evaluation report (see design case example in Chapter 8).

6.7 Sizing of Semicontinuous Units in a Steady State Overall Environment

Several unit operations in a biochemical plant may operate in batch or semicontinuous mode. This creates a problem in sizing equipment when a steady state overall computing environment is assumed as in BioDesigner. To practically solve this problem, a variable called Utilization Factor is used for each operation. The value of the utilization factor (always between zero and one) of a unit operation is equal to the fraction of time during a cycle time that the unit is functioning. More specifically, if $t_c$ is the cycle time of a fermentation plant (e.g., 40 h) and $t_u$ is the time that a unit
operation is on during that cycle time (e.g., 12h), then the utilization factor of that unit is:

\[
\text{Utilization Factor} = \frac{t_u}{t_c} = \frac{\text{Time Unit is On}}{\text{Cycle Time}}
\]

The estimated size of each unit operation, based on averaged steady state overall operation, is divided by its utilization factor to get a more accurate approximation. Utilities consumed by a unit operation are multiplied by its utilization factor to take into account the idle time during which utilities are normally not consumed.

The performance of batch unit operations is averaged over a cycle time and consequently these units are also handled as continuous or semicontinuous units. In the future, the utilization factor will be supplemented with some scheduling capabilities to more accurately predict size of equipment for batch and semicontinuous unit operations. In the future, the utilization factor concept will be supplemented with some scheduling capabilities to more accurately predict size of equipment for batch and semicontinuous unit operations.
Chapter 7

Unit Operations in BioDesigner

7.1 Introduction

As mentioned in Chapter 6, unit operation objects constitute the heart of BioDesigner. Short-cut models are mainly used to describe performance of unit operations for the following reasons: (1) to reduce implementation time; (2) more rigorous models are often not more predictive than the short-cut ones since the main limitation of both is availability and quality of operating data; and (3), BioDesigner is intended to be used for quick, preliminary design. For these reasons the use of short-cut models is adequate. To estimate the purchase cost of equipment, BioDesigner uses functions that were developed by curve-fitting price data from vendors, literature, and engineering companies. What follows is a description of the unit operation models that are used in BioDesigner; equipment purchase cost data are also presented.

7.2 Description of Unit Operation Models

7.2.1 Continuous Heat Sterilizer

The purpose of this model is to rigorously simulate the holding tube of a continuous sterilizer and to approximately estimate the cost of the entire
system, including: the holding tube along with its insulation, the heat exchangers for energy conservation, the pumps, etc. Continuous heat sterilization is simulated assuming logarithmic death rate of microorganisms and spores (Wang et al, 1979)

\[- \frac{dN}{dt} = kN\]

where \(N\) is concentration of viable organisms in number/liter, \(k\) is specific death rate constant in sec\(^{-1}\), and \(t\) is time in minutes. At \(t = t_0\), \(N = N_0\).

The specific death rate \((k)\) is related to sterilization temperature by an Arrhenius type equation

\[k = A \exp\left(\frac{\Delta E}{RT}\right)\]

where \(A\) is the frequency factor in sec\(^{-1}\), \(\Delta E\) is the activation energy of death in cal/mole, \(R\) is the gas constant in cal/mole-\(^0\)K, and \(T\) is the absolute temperature in \(^0\)K. BioDesigner provides good default values for \(A\), \(\Delta E\), \(R\), and \(T\), which the user has the option to modify.

Because actual plug flow through the holding tube of a continuous sterilizer is never achieved, an axial dispersion model is assumed to account for residence time distribution. Solving the material balance equation, one obtains

\[\frac{N}{N_0} = \frac{4\delta \exp\left(\frac{N_{pe}}{2}\right)}{(1+\delta)^2 \exp\left(\frac{N_{pe} \delta}{2}\right) - (1-\delta)^2 \exp\left(\frac{N_{pe} \delta}{2}\right)}\]

where

\[
\delta = \sqrt{1 + \frac{4N_R}{N_{pe}}}, \quad N_R = \frac{KL}{U}, \quad N_{pe} = \frac{UL}{D_z}
\]
U is the average medium velocity (m/sec), L is the length of the holding tube (m), \( \text{Dz} \) is the axial dispersion coefficient (m\(^2\)/sec). \( \text{Dz} \) is estimated as a function of the Reynolds number from the following equation

\[
\frac{\text{Dz}}{\mu/\rho} = 6.0936 \times 10^5 - 1.2324 \times 10^5 \ln(\text{Re}) + 6279.2 \left[ \ln(\text{Re}) \right]^2
\]

where \( \mu \) is the liquid viscosity (kg/m-s) and \( \rho \) is the liquid density (kg/m\(^3\)). This equation was derived by curve fitting experimental data (Figure 40, Levenspiel, 1972).

**Solution algorithm.** In the rate mode of calculation, the tube diameter \( (d_t) \) and length \( (L) \) are given and the equations are solved to estimate the sterility level \( (N/No) \). In the design mode of calculation, the tube diameter \( (d_t) \) and the sterility level \( (N/No) \) are given and the equations are solved iteratively to estimate the tube length \( (L) \).

The size estimation of heat exchangers that are used for energy conservation is based on intermediate and final temperatures of various streams that are specified by the user.

### 7.2.2 Bioreactors

The purpose of bioreactor models is to simulate any type of biochemical reaction and to carry out material and energy balances around such systems. The bioreactor models in the current version of BioDesigner are simple stoichiometric reactors. The user provides the mass stoichiometric coefficients \( (A_i) \) of the various components for the overall reaction and the
extent of reaction (x). The following algorithm is used to estimate the material balances.

First, the limiting component is identified. Its outlet mass flowrate ($F_{out}$) as a function of its inlet mass flowrate ($F_{in}$) and the extent of reaction (x) is:

$$F_{out} = F_{in} (1.0 - x)$$

Second, the outlet mass flowrate ($F_{out_i}$) of component (i) as a function of its inlet mass flowrate ($F_{in_i}$), the extent of reaction (x), and the mass stoichiometric coefficients ($A_i$), is given by the following equation:

$$F_{out_i} = F_{in} (1.0 - x \frac{A_i}{A_k})$$

where $A_k$ is the mass stoichiometric coefficient of the limiting component.

Bioreactor volume ($V$) for continuous operation is estimated based on the overall feed flowrate ($F$) and the dilution rate ($D$):

$$V = \frac{F}{D}$$

For batch operation, reactor volume estimation is based on the amount of broth required to be produced per cycle.

Agitation power estimation is based on the unit power requirement (kW/m$^3$ of broth) that the user specifies.

Heat release $Q_f$ (kcal/L-hr) due to oxidation is estimated based on the oxygen uptake rate $Q_{O_2}$ (mmoles/L-hr) (Cooney et al., 1968).
\[ Q_t = 0.12 \, Q_0 \]

To estimate the purchase cost of equipment, different functions are used for the various types of bioreactors. BioDesigner currently has cost data for stirred tank, air-lift, fluidized bed, and plug flow bioreactors.

### 7.2.3 Disc-Stack Centrifuge

The purpose of this model is to simulate a disc-stack centrifuge for solid-liquid separation.

Separation by disc-stack centrifugation is based on the sedimentation principle. The design of centrifugal separators has been analyzed successfully by using the Sigma theory (Ambler, 1952, 1961; Frampton, 1963; Murkes and Carlson, 1978; Axelsson, 1985). According to this analysis, to separate from a dispersion all particles of diameter greater than or equal to a limit particle diameter (\(d_{\text{lim}}\)), the maximum separator capacity (throughput) is given by the following equation.

\[
Q = \eta \left[ \frac{d_{\text{lim}}^2 \Delta \rho \, g}{18 \mu} \right] \left[ \frac{2 \pi}{3g} \, \omega^2 \, N \, \cot \alpha \, (r_1^3 - r_2^3) \right]
\]

where \(\eta\) is the efficiency of the centrifuge (actual throughput/theoretical throughput), \(d_{\text{lim}}\) is the equivalent Stokes' diameter of the limit particle, \(\Delta \rho\) is the density difference between the solid particles and the liquid, \(\mu\) is the viscosity of the liquid, \(\omega\) is the angular speed of the discs, \(N\) is the number of discs, \(\alpha\) is the angle between the discs and the axis of the centrifuge, \(r_1\) and \(r_2\) are the outer and inner diameter of the discs respectively, and \(Q\) is the
maximum capacity for complete removal of limit particles. The throughput of a centrifuge is proportional to the density difference between the solid particles and the liquid and proportional to the square of the limiting particle diameter. Therefore, large and dense particles are separated more easily than small and light ones.

The term in the second pair of brackets of the equation has units of square meters and is called the Sigma factor. The Sigma factor of a centrifuge specifies its size as the equivalent surface area of a sedimentation tank required to achieve the same separation results as the centrifuge.

The case where $\eta = 1$ would describe performance of a centrifuge based on Stokes' law. In reality, however, deviations from Stokes' law (i.e. nonspherical particles, non-Newtonian rheology, hindered settling, non-laminar flow, etc.) result in lower values of $\eta$. It has been found by experience that the efficiency of disc-stack separators is usually less than 50% with an average value of about 30%.

In "Design Mode" of calculation, the user specifies the design component that needs to be fully recovered and the model estimates the required size of the centrifuge (its Sigma factor) using the above equation. In "Rate Mode" of calculation, the user specifies the Sigma factor of the centrifuge and the model estimates the recovery of each component.

**7.2.4 Microfilter**

The purpose of this model is to simulate a tangential flow microfilter used for solid-liquid separation and to estimate its capital and operating cost.
The type of filtration, most frequently used for cell harvesting, is tangential flow microfiltration. In this kind of filter, cross flow parallel to the filter surface is used to enhance filtration rate. The pore sizes of microfilters usually range from 0.1 to 0.45 microns.

Separation by microfiltration is essentially a fractionation technique by which particles are separated on the basis of their physical size. However, it should be noted that this is only a very simplistic view; separation is affected both by chemical and physical interactions between the materials that pass through the membrane and the membrane itself and their interactions with the solvent.

According to the gel polarization and lateral migration model (Belfort and Altena, 1985), the flux of the permeate for hollow-fiber tangential flow microfilters is given by the following two terms:

Diffusive term
\[ D_t = 1.295 \left( \frac{D^2}{r L} \right)^{0.33} \ln \left( \frac{C_w}{C_b} \right) U^{0.33} \]

Lateral migration term
\[ L_t = 5.0 \left( \frac{\alpha}{r} \right)^2 \left( \frac{a}{V} \right) \left[ 1 - \frac{r'}{r} \right] U^2 \]

where \( D_t \) is the diffusive flux (m/s), \( D \) is the diffusivity (m\(^2\)/s), \( r \) is the tube radius (m), \( L \) is the tube length (m), \( C_w \) is the concentration of particles on the wall (g/l), \( C_b \) is the bulk particle concentration (g/l), \( U \) is the average axial velocity of the fluid (m/s), \( \alpha \) is the particle radius (m), \( L_t \) is the lateral term of flux (m/s), \( v \) is the kinematic viscosity (m\(^2\)/s), and \( r^* \) is the
equilibrium radius position (m). Wall particle concentration \(C_w\) can take values up to 95% v/v (Belfort and Altena, 1985). Diffusivity is calculated by the following equation:

\[ D = 0.025 \alpha^2 \gamma \]

where \(\gamma[1/s]\) is the wall shear rate and is given by the following equation:

\[ \gamma = \frac{8}{2} \frac{U}{r} \]

For cell harvesting, the diffusive term is the dominating term. Therefore, the permeate flux is almost independent of particle size. The dependence of flux on the ratio \(C_w/C_b\) suggests plug flow operating mode. This can be achieved to some extent by arranging many filters in series. High axial velocities are also beneficial because they reduce the concentration polarization.

In large-scale operation, the permeate flux for cell harvesting usually ranges between 10 and 50 l/m²-h.

7.2.5 Ultrafilter

The design of ultrafilter units is based on the gel polarization model (Flaschel et al. 1983). According to this model, for a protein solution, the flux of solvent through the membrane is given by the following equation:

\[ J = k_d \ln \frac{C_{im} - C_{if}}{C_{ib} - C_{if}} \]

where \(J\) [ms⁻¹] is the solvent flux, \(k_d\) [ms⁻¹] is the mass transfer coefficient, and \(C_{im}, C_{ib}, C_{if}\) [mol/m³] are the solute concentration on the membrane,
in the bulk phase, and in the filtrate, respectively. For quantitative retention (C_{IF}=0), the above equation is simplified to:

\[ J = k_d \ln \frac{C_{im}}{C_{ib}} \]

If the solute concentration on the membrane reaches the saturation concentration, C_{im} remains constant even if the bulk phase concentration is further increased:

\[ J = k_d \ln \frac{C_{is}}{C_{ib}} \]

where C_{is} [mol/m^3] is the solubility saturation concentration. C_{is} can take values up to 0.95 v/v (Belfort and Altena, 1985). To estimate the mass transfer coefficient (k_d) at different hydrodynamic conditions, the general boundary layer theory is applied. For tubular membranes, this theory gives the following results for laminar and turbulent flow regimes, respectively:

**Laminar Flow:**  \[ k_d = 1.62 \left( \frac{u D_i^2}{d_i l_i} \right)^{1/3} \]

**Turbulent Flow:**  \[ k_d = 0.023 \frac{u^{0.8} D_i^{0.67}}{d_i^{0.2} \nu^{0.47}} \]

where u [m/s] is the superficial fluid velocity in the flow channel along the membrane, D_i [m^2/s] is the diffusion coefficient of solute i, d_t [m] is the diameter of the tubular membrane, l_t [m] is the length of the tubular membrane and \( \nu \) [m^2/s] is the kinematic viscosity. D_i can be measured
experimentally or calculated from Stokes-Einstein's equation (Colton and Lowrie, 1981).

The flux rates of large-scale ultrafilter units usually range from 10 to 50 L/m²/h depending on the specific device, the operating conditions, and especially the properties of the material being processed. The protein solutions typically contain a variety of low and high molecular weight solutes that hinder the solvent flux through the membrane. Fortunately, a large fraction of the low molecular weight solutes pass through the membrane, contributing to product purification.

Ultrafiltration membranes need to be cleaned regularly in order to maintain high flux and longevity. The average life of ultrafiltration membranes strongly depends on the degree of utilization and the operating conditions and ranges approximately from 6 to 12 months (or 1000 to 2000 hours of operation time).

Ultrafiltration competes in industry with reverse osmosis and evaporation for concentration and purification of product solutions. Until only recently, reverse osmosis was the least utilized in the bioprocess industries. However, it has considerable potential for the concentration of medium molecular weight (100 - 5,000 MW) product solutions. Evaporation has been the traditional method but mainly for non-proteinaceous products. It is usually performed in multi-stage vacuum evaporators where the temperature is kept as low as possible. For heat sensitive products, rotary vacuum evaporators are used with very short residence times (a few seconds). Ultrafiltration is a relatively new technique with promising comparative advantages. It can operate at low temperature (5 °C), thus keeping the activity loss due to thermal degradation at a minimum. It also
has the advantage that substances of low molecular weight (less than 10,000) pass through the membrane and contribute to product purification.

7.2.6 Rotary Vacuum Filter

Rotary vacuum filtration is used extensively for biomass removal (for extracellular products), biomass recovery (when biomass is the product), and recovery of product precipitates crystals.

A filter cloth covers a rotating drum. Pressure outside the drum is atmospheric, but pressure inside the drum is a partial vacuum. The drum is partially submerged in the solution and rotates at low speed during operation. Liquid is sucked through the filter cloth and solids are retained on the surface of the drum, forming cake. When the cake rotates out of the liquid, it is washed, dried, and removed. The filter cloth is often precoated (e.g., with diatomaceous earth) to facilitate filtration and clarification. Addition of filter aids to the broth further enhances filtration by causing colloids in the broth to adsorb on particles of filter aid, effectively counteracting two of the chief problems of rotary vacuum filtration. The first problem, particularly with mycelial broths, is the compressibility of the accumulated biomass, which dramatically reduces its permeability. The filter aids produce a much less compressible cake. The second problem is the penetration of small particles, such as fragmented mycelia or bacterial cells, into the precoat layer of the filter. This penetration can blind the pores of the precoat matrix.

The theory of rotary vacuum filtration is based on Darcy's law (Belter et al., 1988). If the filtration resistance of filter medium is negligible
compared to the resistance of cake, then the flux \((J)\) for compressible cake is given by the following equation:

\[
J = \left[ \frac{2 \beta (\Delta p)^{1+s}}{\mu \alpha' \rho_o t_c} \right]^{1/2}
\]

where \(\beta\) is the fraction of time that the filter is submerged, \(\Delta p\) is the pressure drop across the cake, \(s\) is the cake compressibility, \(\mu\) is the liquid viscosity, \(\alpha'\) is a constant related largely to the size and shape of the particles forming the cake, \(\rho_o\) is the mass of cake solids per volume of filtrate, and \(t_c\) is the cycle time of the filter. For incompressible cake, \(s = 0\), and \(\alpha' = \alpha\) (\(\alpha\) is the specific cake resistance). For biomass removal, \(s\) ranges from 0.1-0.8.

Knowing the filtrate flux, the filter area \((A)\) is estimated by the following equation:

\[
A = \frac{V}{J}
\]

where \(V\) is the feed flowrate to the filter. Instead of allowing the model to specify the flux, the user can specify its value if it is known.

### 7.2.7 Basket Centrifugal Filtration

In biochemical processes, centrifugal filtration is mainly used for dewatering and washing of crystalline and fibrous solids. This method uses filtration driven by centrifugal force. The equipment consists of a rotor with a perforated cylindrical shell, lined with an appropriate filter medium, mounted on a bowl bottom, and surmounted by an annular lip ring to retain its contents.
The geometry of a centrifugal filter is shown in Figure 7.1. $R_o$ is the radius of the porous cylinder. The feed solution is driven against the inner wall of the cylinder by rapid rotation. The surface of this solution is located at radius $R_1$, which is kept constant by continuous feed. Cake accumulates on the wall; the cake's interface is located by $R_c$.

A centrifugal filter can operate either in batch mode or continuously. The operating cycle of a batch centrifugal filter involves the following steps: loading, washing, dry spinning, and unloading of cake. Cake can be unloaded either manually or automatically. A continuous centrifugal filter makes use of a knife-discharge mechanism for continuous cake removal (Ambler, 1988).

![Figure 7.1: Schematic representation of a centrifugal basket filter](image)

The theory of centrifugal filtration is also based on Darcy's law (Belter et al., 1988). It is assumed that the filtration resistance of filter medium is negligible compared to the resistance of cake and that the cake is incompressible. BioDesigner contains two models for centrifugal filtration, one for continuous filtration and another one for batch.
**Continuous centrifugal filtration.** The filtrate flux is given by the following equation (Belter et al., 1988):

\[
J = \left[ \frac{\omega^2 \rho}{2 \, \mu \, \alpha \, \rho_o} \right] \frac{(R_o^2 - R_e^2)}{\ln (R_o / R_e)}
\]

where \( \omega \) is the angular velocity of the cylindrical shell, \( \rho \) is the density of the liquid, \( \mu \) is the liquid viscosity, \( \alpha \) is the specific resistance of the cake, and \( \rho_o \) is the mass of solids per volume of liquid feed. Based on filtrate flux, the model estimates the size and number of centrifugal filters required. Instead of specifying the parameters of the above equation, the user can provide the value of filtrate flux \( (J) \). For crystalline incompressible solids, filtrate flux is usually in the thousands of L/m²-h. Dimensions of continuous centrifugal filters are usually in the following range: \( (R_o) \) from 25 - 75 cm and \( (l) \) from 25 - 100 cm.

**Batch centrifugal filtration.** For batch filters, the user specifies the geometry of the unit and the model estimates the filtration time \( (t_f) \):

\[
t_f = \frac{\mu \, \alpha \, \rho_e \, R_e^2}{2 \, \rho \, \omega^2 \, (R_o^2 - R_e^2)} \left[ \left( \frac{R_o}{R_e} \right)^2 - 1 - 2 \ln \left( \frac{R_o}{R_e} \right) \right]
\]

where \( \rho_c \) is the mass of solids per volume of cake. The user also specifies the total downtime that includes time for loading, washing, unloading, and cleaning. The volume of feed material processed during one batch is approximately equal to:

\[
V = \pi \left( \frac{\rho_c}{\rho_o} \right) l \, (R_e^2 - R_c^2)
\]
where $l$ is the height of the centrifuge. The averaged feed flowrate that can be handled during one batch is $V/t_c$ ($t_c$ is the total cycle time). The number of units is estimated by comparing the above averaged feed flowrate to the actual averaged feed flowrate towards this process step. Dimensions of batch centrifugal filters are usually in the following range: $R_o$ from 6 - 125 cm and $l$ from 4 - 50 cm.

7.2.8 Cell Disruption

Although a number of bench scale cell disruption techniques have been reported in the literature, only high pressure homogenization and bead milling are used on the large scale. Osmotic shock sometimes also is used for periplasmic products.

7.2.8.1 High Pressure Homogenizer

High pressure homogenization is the most common cell disruption technique used on the large scale. The cell slurry is pumped through a narrow ring gap at pressures up to 1200 atm (depending on the microorganism and the operating mode). The large pressure drop creates high liquid velocities that cause cavitation and turbulence. The resulting fluid oscillation and high shear forces are the primary causes of cell disruption. The disrupted cells release the intracellular protein but because of the high shear rate a fraction of the protein is denatured. To increase the extent of cell disruption, the broth typically passes several times through
the disruption unit. The multiple passes also serve to reduce the suspension viscosity by fragmenting the released nucleic acids.

Cell disruption is described by an empirical model, which relates the fraction of cells disrupted (R) to the pressure drop (∆p) across the valve and the number of discrete disrupter passes (N) (Hetherington et al. 1971).

\[ R = 1 - \exp(-k \Delta p^\alpha N) \]

Where \( \alpha \) is a constant (cell- and equipment-dependent) and \( k \) is a release rate constant (cell-, equipment-, and temperature-dependent). Constant \( \alpha \) for most microorganisms is approximately equal to 2.9. The fraction of the released intracellular proteins that are denatured (D) is given by the following equation:

\[ D = 1 - \exp(-\phi k \Delta p^\alpha N) \]

the parameter \( \phi \) usually has values between 0.0 and 0.3.

7.2.8.2 Bead Mill

The mechanism of cell disintegration by bead mill is based on the concussion of glass (or steel) beads on the cell surfaces. A bead mill has a horizontal chamber into which cell suspensions can be fed in either a batch or continuous mode. The chamber is filled (up to 80-85% of the chamber volume) with glass beads of a fixed diameter which ranges from 0.1 to 3 mm. A rapidly rotating shaft (2,000 - 6,000 RPM) is located in the center of the chamber and is fitted with discs. The rotation of the discs causes the
grinding beads to move in a circular manner in the chamber. The kinetic energy transferred from the beads creates impact and shear forces between the individual beads and between the beads and the microbial cells.

The rates of product release and denaturation are described by the following equations:

\[
\text{Product Release: } R = 1 - \exp(-k\,t) \\
\text{Product Denaturation: } D = 1 - \exp(-\phi\,k\,t)
\]

where \(k\) is a kinetic constant, \(t\) is the average residence time of broth in the chamber (usually around 2 min), and \(\phi\) is the denaturation parameter.

### 7.2.9 Chromatography

As explained in Chapter 3, chromatography is mainly used in the final stages of downstream processing to complete the purification of biological products that are required in high purity.

The main objective of the chromatography models in BioDesigner is to estimate the number and size of columns required to carry out the desired purification. Most parameters affecting purification and overall material balances are specified by the user.

#### 7.2.9.1 Ion Exchange

The user provides the following information:
Total cycle time, $t_c$ [h].
Total binding capacity of resin, $C$ [g/L].
Column length, $L$ [m].
Maximum column diameter, $D_{max}$ [L].
Binding fraction of component $i$, $F_i$ [g/L].
Product recovery yield, $Y$.
Product purification factor, $PF$.
Concentration factor, $CF$.
Overdesign factor, $ODF$.

The total cycle time is the sum of the loading, washing, elution, and regeneration times. The total binding capacity of the resin refers to all the proteins that are present in the feed stream under flow and mobile conditions identical to feed stream. The binding capacity is estimated experimentally or approximately from Scopes' correlation, providing the molecular weight of the main components is known (Scopes, 1982; p.105, Fig. 5.3).

If $Q$ [L/h] is the averaged feed flowrate to the unit and $C_i$ is the concentration of component $i$ in the feed stream, then the volume, $V$, of the column is:

$$V = \frac{Q t_c \sum_{i=1}^{k} C_i F_i}{C} \times ODF$$

where $k$ is the total number of components in the feed stream. The diameter, $D$, of the column is:

$$D = \left[ \frac{4 V}{\pi L} \right]^{1/2}$$

If $D > D_{max}$, then multiple columns are assumed in parallel.
The amount of product in the outlet stream is estimated using the recovery yield. The amounts of other components in the outlet stream are estimated to satisfy the purification factor requirement:

\[
PF = \frac{(\text{Product})_{\text{OUT}}}{(\text{Product} + \text{Contaminants})_{\text{OUT}}} \cdot \frac{(\text{Product})_{\text{IN}}}{(\text{Product} + \text{Contaminants})_{\text{IN}}}
\]

This is an iterative procedure that involves estimation of appropriate amounts of each component (contaminant) that is removed. The amount of solvent (usually water) in the outlet stream is estimated using the concentration factor, CF.

The user also specifies the frequency of resin replacement and the unit cost of resin. Finally, the volumes of liquid for elution, washing, and regeneration along with their unit costs are also specified by the user.

The current chromatography models in BioDesigner have limited predictive capabilities. A combination of experimental data and modeling is required to accurately size a chromatography unit.

7.2.9.2 Affinity Chromatography

The algorithm used to size an affinity chromatography column is identical to that used for ion exchange. The only difference is in the default values of some variables.

7.2.9.3 Gel Filtration

The user provides the following information:
Linear flowrate, \( U \) [m/h].
Column length, \( L \) [m].
Maximum column diameter, \( D_{\text{max}} \) [m].
Sample volume per injection, \( B_{\text{SAM}} \) (% of column volume).
Sample frequency, \( F_{\text{SAM}} \) (% of column length).
Recovery yield, \( Y \).
Purification factor, PF.
Dilution factor, DF.
Overdesign factor, ODF.

Explanation: Sample frequency 30% means that a new sample is injected when the previous one has moved 30% along the column length.

The injection interval (time between two consecutive injections), \( t_{\text{inj}} \), is equal to:

\[
t_{\text{inj}} = \frac{F_{\text{SAM}}}{100} \times \frac{L}{U}
\]

If \( Q \) [L/h] is the averaged feed flowrate to this unit, then the amount of material fed and processed during one injection interval must be equal to one sample volume, that is:

\[
Q\ t_{\text{inj}} = \frac{B_{\text{SAM}}}{100} \ V
\]

or

\[
V = \frac{100\ Q\ t_{\text{inj}}}{B_{\text{SAM}}}
\]

where \( V \) is the volume of the column. The value of the volume is multiplied by the overdesign factor for conservative design.

The required length, \( L \), of the column is estimated experimentally using an analytical column. The diameter, \( D \), of the column is:

\[
D = \left[ \frac{4\ V}{\pi\ L} \right]^{1/2}
\]

If \( D > D_{\text{max}} \), then multiple columns are assumed in parallel.
To complete the material balances, the same procedure as in the case of ion exchange is applied.

7.2.10 Distillation

Distillation is used for recovery and purification of volatile compounds, such as ethanol and organic acids.

BioDesigner uses a short-cut model for distillation that estimates the number of theoretical stages. The user specifies the recovery fraction of each component in the distillate. Based on this information, the program estimates the overall material balances.

The user provides the relative (to the heavy key component) volatilities (α_i) of the various components. The program looks for α_i = 1, then designates component (i) as the heavy key and component (i-1) (the component with the next higher relative volatility) as the light key.

The minimum reflux ratio (R_{min}) is estimated using Underwood's (1948) correlation:

\[ R_{min} = \left[ \sum_{i=1}^{k} \frac{\alpha_i X_{Di}}{\alpha_i - \theta} \right] - 1 \]

where k is the number of components, X_{Di} is the mole fraction of component i in the distillate, and θ is determined by trial and error, using the following equation:
\[ 1 - q = \sum_{i=1}^{k} \frac{\alpha_i x_i}{\alpha_i - \theta} \]

where \( X_{Fi} \) is the mole fraction of component \( i \) in the feed and \( q \) is the quality of the feed, defined as

\[ q = \frac{H_{DP} - H_F}{H_{BP} - H_F} \]

where \( H_{DP} \) = enthalpy of the feed as saturated vapor,
\( H_{BP} \) = enthalpy of the feed as saturated liquid,
\( H_F \) = enthalpy of the feed.

After finding \( R_{\text{min}} \), the program calculates the minimum number of stages \( N_{\text{min}} \), using the Fenske (1932) equation:

\[ N_{\text{min}} = \log \left[ \left( \frac{x_i}{x_h} \right)_D \left( \frac{x_h}{x_i} \right)_B \right] \left[ \frac{1}{\log \alpha_i} \right] \]

where the subscripts \( l \) and \( h \) denote the light and heavy key components while \( D \) and \( B \) denote distillate and bottom.

The user also specifies the desired reflux ratio \( R \) as a fraction of \( R_{\text{min}} \) (\( R \) is usually 10-50% greater than \( R_{\text{min}} \)). With this additional input, the program calculates the number of theoretical stages \( N \) using Gilliland's (1940) correlation as expressed by Eduljee (1975):

\[ \frac{N - N_{\text{min}}}{N + 1} = 0.75 - 0.75 \left[ \frac{R - R_{\text{min}}}{R + 1} \right]^{0.5668} \]
Alternatively, the user can input the desired value of \( N \), and the program can find \( R \), using the above equation.

### 7.3 Equipment Purchase Cost Data

This section provides approximate purchase equipment cost data for several unit operations used in BioDesigner. The data come from cost estimating articles or books and vendor quotations. All costs were factored to a June 1988 basis, or a *Chemical Engineering* index number of 341.6. These data are used in BioDesigner to estimate the total purchase cost of equipment. The other items of the total capital investment for a new plant are estimated as a function of the total purchase cost of equipment (using multipliers).
Figure 7.2: Stirred Fermentor, 316 Stainless Steel
Source: Literature and Vendor Information
Figure 7.3: Disc-Stack Centrifuge.
Source: Westfalia Separator AG
Heinz-Georg Kronchen
Postfach 3720, D-4740 Oelde
West Germany
Figure 7.4: High Pressure Homogenizer.
Source: APV Gaulin, Inc.
Everett, MA 02149-4512
Figure 7.5: Bead Mill Cell Disrupter.  
Source: Glen Mills, Inc.  
203 Brookdale Street  
Maywood, N.J. 07607
Figure 7.6: Ultrafiltration Hardware.
Source: Kalk and Langlykke (1985)
Figure 7.7: Rotary Vacuum Filter.
Source: Perry's Chemical Engineers' Handbook
Sixth Edition, p.19-89, Fig.19-116.
(Material: stainless steel)
Figure 7.8: Basket Centrifugal Filter.
Source: Perry's Chemical Engineers' Handbook
Sixth Edition, p.19-101, Fig.19-127.
The data are for 'Underdriven' Centrifuges;
for 'Top Suspended' multiply by 2.3.
Figure 7.9: Chromatography Equipment.
Source: Amicon Corporation
72 Cherry Hill Dr.
Beverly, MA 01915

Material of Construction

Low Pressure Column: Plastic
High Pressure Column: Stainless Steel
(It can be used for HPLC)

Cost of Peripherals ($) (Pumps, Valves, Control Equipment, etc.)

<table>
<thead>
<tr>
<th>Throughput (L/min)</th>
<th>Low Pressure Column</th>
<th>High Pressure Column</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 - 2.5</td>
<td>83,000</td>
<td>140,000</td>
</tr>
<tr>
<td>2.5 - 5.0</td>
<td>102,000</td>
<td>185,000</td>
</tr>
<tr>
<td>5.0 - 20.0</td>
<td>111,000</td>
<td>230,000</td>
</tr>
</tbody>
</table>
Chapter 8

Illustrative Example

8.1 Background: Production of Porcine Somatotropin

Porcine somatotropin (pST) (growth hormone) accelerates growth rate and improves carcass quality when administered to pigs (Snoswell, 1988; Vize and Wells, 1987). One dose of about 200 mg pST is required per pig-life. Porcine growth hormone is a relatively small protein molecule (MW = 20,000 Daltons) and is formed in minute amounts in the pituitary gland. Consequently, isolation and extraction of pST from animals is expensive, time consuming, and limited to the quantities which can be obtained from the pituitary glands of slaughtered animals. However, the gene for pST synthesis has been isolated and expressed in E.coli (20-30 % per total cell protein) to permit production in fermentation for commercial use. PST is formed in bacterial cells as an inactive inclusion body requiring solubilization and refolding to its native structure. This along with the high purity requirement result in low total recovery yield (less than 30%).

8.2 Level 1: Input-Output Analysis

8.2.1 Design Basis
The conceptual design of the plant in this case study is based on a pST demand for 30 million pigs per year. With one dose per pig-life of 200 mg, purified pST production is 6,000 kg/yr. Using Table 3.2 as a guide (or the Recovery Yield estimation item of the Input-Output Analysis facility of BioDesigner) an overall recovery yield of 30% is assumed. Therefore, the amount of pST that must be produced in the fermentor is 20,000 kg/yr. The plant is assumed to operate 330 days/year with its upstream section working around the clock and its downstream section working 1 shift/day.

8.2.2 Raw Materials Amount and Cost Estimation

The Elemental Balance facility of BioDesigner (see Appendix III for more details and an example) is used to estimate the amount of raw materials required for the production of 20,000 kg/yr of pST. It is assumed that glucose is used as the carbon and energy source and gaseous ammonia as nitrogen source. Assigning molecular formulas to pST and biomass (non-pST containing biomass), the overall reaction that takes place in the fermentor is described by the following equation:

\[
\text{Glucose + Oxygen + Ammonia} \rightarrow pST + \text{Biomass + Carbon Dioxide + Water}
\]

\[
\text{CH}_2\text{O} + a\text{O}_2 + b\text{NH}_3 \rightarrow d\text{CH}_{1.58}\text{O}_{0.34}\text{N}_{0.29} + e\text{CH}_{1.80}\text{O}_{0.5}\text{N}_{0.2} + f\text{CO}_2 + h\text{H}_2\text{O}
\]

Assuming that total cell protein constitutes 60% of total dry cell mass and pST constitutes 20% of total cell protein, the Elemental Balance facility of BioDesigner (see Appendix III) yields:
<table>
<thead>
<tr>
<th>Compound</th>
<th>Consumption/Production</th>
<th>Cost/Price</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg/yr</td>
<td>$/kg</td>
</tr>
<tr>
<td>Glucose</td>
<td>333,330</td>
<td>0.4</td>
</tr>
<tr>
<td>Oxygen</td>
<td>127,290</td>
<td>0.0</td>
</tr>
<tr>
<td>Ammonia</td>
<td>24,530</td>
<td>0.3</td>
</tr>
<tr>
<td>pST</td>
<td>20,000</td>
<td>10,000.0</td>
</tr>
<tr>
<td>Biomass</td>
<td>146,670</td>
<td>0.0</td>
</tr>
<tr>
<td>H2O</td>
<td>130,080</td>
<td>0.0</td>
</tr>
<tr>
<td>CO2</td>
<td>188,420</td>
<td>0.0</td>
</tr>
</tbody>
</table>

The economic potential at this level of design, taking into account only the value of pST and the cost of glucose and ammonia, is:

$$\left[ \frac{\text{Economic Potential}}{\text{(Economic Potential)}} \right] = \frac{(0.3)(20,000)(10,000) - (333,330)(0.4) - (24,530)(0.3)}{(0.3)(20,000)(10,000)} = 0.998$$

The assumed selling price of pST comes from a cost-benefit analysis (Snoswell, 1988). At this level the project seems very attractive; fermentation media cost is only 0.2% of revenue. This is typical of high value biological products. The selling price of such products, if there is a proprietary position, is determined by product value and is independent of manufacturing cost. Second, for such products required in high purity, most of the production cost may occur in the downstream section.

### 8.3 Level 2: Upstream Section

#### 8.3.1 Synthesis of Upstream Section
The selection and ordering of unit operations for the upstream section is done using the synthetic component of BioDesigner. Of the upstream section synthesis rules evaluated, the ones paraphrased below were used:

1. *Is number of reaction steps required for product formation less than 3?* Enzyme reactors are considered for product formation only if the number of reaction steps required for product formation is less than 3. In this case, the answer is no because the product is a protein and a large number of reaction steps are required for its formation.

2. *What is the type of product (Biomass or a Molecule)?* This rule precludes the use of immobilized cell reactors if Biomass is the product type.

3. *What is the product location (Intracellular or Extracellular)?* If the product location is Intracellular, as in this case, then a cell reactor with growing cells should be used for product formation.

4. *What is the type of microorganism (Bacterium, Yeast, or Animal Cell)?* If the type of microorganism is Bacterium, as in this case, then only stirred tank bioreactors are considered.

5. *Is the microorganism aerobic?* If the microorganism is aerobic, then a compressor is used for aeration.

6. *Is gaseous ammonia used as nitrogen source?* If the answer is yes and
a compressor is used for air supply, then a mixer is included to mix the ammonia stream with the air stream.

7. *Is the microorganism recombinant?* If the answer is yes, then an absolute outlet air filter for the fermentor is included to prevent any microorganisms from escaping to environment. An acid treatment cycle is also included to kill the cells in the fermentor after the end of fermentation.

8. *Is the use of a continuous sterilizer desired?* This question is asked if the type of the microorganism is Bacterium or Yeast. If animal cells are used for product formation, then a microfilter is assumed for media sterilization.

The result of the upstream synthesis session is identical to the flowsheet shown in Figure 3.1. Tank BLT101 is used for media preparation; the media are sterilized by the STR101 continuous sterilizer. The S105 stream is for ammonia supply. The CMP101 compressor is used for air supply. The M2X101 mixer mixes the ammonia with the air stream before they pass through the absolute inlet air filter (AFL101). FRM101 is the production fermentor. The BLT102 tank is a holding tank that isolates the upstream from the downstream section.

### 8.3.2 Analysis of Upstream Section

The analysis of the upstream section starts with the initialization of the flowsheet. First, the chemical components along with some of their
properties are specified. Effort is made to lump together components that do not play a major role in the analysis. For example, the various salts (MgSO₄, NH₄CL, K₂SO₄, KH₂PO₄, Na₂HPO₄, etc.) shown in Table 8.1 (detailed composition of fermentation media) are lumped into one component called SALTS. Next, the flowrates of the various components in the feed streams are specified. Finally, the various unit operations are initialized by providing engineering specifications.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>86.0</td>
</tr>
<tr>
<td>NH₃ (gaseous)</td>
<td>5.6</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>1.5</td>
</tr>
<tr>
<td>NH₄CL</td>
<td>1.9</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>1.6</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.8</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>2.8</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0.031</td>
</tr>
<tr>
<td>Antifoam</td>
<td>0.4</td>
</tr>
<tr>
<td>Trace Elements</td>
<td></td>
</tr>
</tbody>
</table>

Information provided during initialization of unit operations included: **Fermentor:** Fermentation time of the production fermentor is 18 h, based on pilot plant data (Snoswell, 1988). Turnaround time (time required between each batch to empty, clean and sterilize the fermentor, fill it with new sterilized media, inoculate, etc.) is 12 h. Therefore, the cycle time is 30 h. The liquid volume of the production fermentor is 70% of total to account for volume expansion due to aeration and foaming. The aeration rate is 1 VVM (1 Volume of air per Volume of broth per Minute). The maximum required agitation rate is 3 kW/m³. The fermentation temperature is 37 °C. Towards the end of fermentation, the temperature is raised to 42 °C for 40 min to
induce pST synthesis (the pST gene is cloned with a temperature inducible promoter). Several of the above parameters (for instance, the turnaround time, the fermentation temperature, the liquid to total fermentor volume ratio, etc.) constitute default values of BioDesigner that are simply checked by the user during initialization. **Sterilizer:** The continuous sterilizer is sized to fill the production fermentor in 4-5 h. It is designed to allow the residence time of the liquid in the holding section to vary between 1 and 3 min and the sterilization temperature to vary between 135 and 150 °C. **Compressor:** The compressor is sized to provide air to production fermentor(s) at a rate of 1 VVM and at a discharge pressure of 6 barg.

At the end of fermentation, the cells are killed by acid treatment (1 h residence time in the fermentor). Acid treatment also breaks DNA molecules to smaller pieces. The cells alternatively can be killed using heat or other chemical means. During acid treatment, the fermentor is cooled down by passing chilled water through the jacket. The cooling is completed (5 °C final temperature) in the hold-up tank (BLT102). A heat exchanger may also be used to facilitate broth cooling during its transfer (which takes about 2 h) from the fermentor to the holding tank.

After the completion of flowsheet initialization, BioDesigner carries out the material and energy balances, estimates the size and cost of equipment, and carries out an economic evaluation. The annualized cost of the upstream section is $7,202,000. Therefore, the economic potential of the project at this level becomes:

\[
\text{Economic Potential}_2 = \frac{(0.3)(20,000)(10,000) - (7,202,000)}{(0.3)(20,000)(10,000)} = 0.88
\]
The project still appears very attractive. The annualized cost of the upstream section is only 12% of the annual revenue. Economic evaluation criteria that make use of the capital investment also can be applied at this level. For example, the IRR after taxes is 0.74 and the Payback Time is only 0.22 years.

8.4 Level 3: Downstream Section

8.4.1 Synthesis of Downstream Section

BioDesigner uses another knowledge base to synthesize the downstream section. However, all information received during the synthesis of the upstream section becomes available to the system during the synthesis of the downstream section. Of the downstream section synthesis rules evaluated, the ones paraphrased below were used:

1. *What is the location of product (Intracellular or Extracellular)?* If the product is Intracellular, as in this case, cell harvesting should be considered as the first step of downstream processing. Cell harvesting reduces the broth volume and gets rid of extracellular impurities and other undesirable components (e.g., proteases that cleave product protein).

2. *What is the density and the average diameter of the microorganism?* If the density of the microorganism is greater than 1050 kg/m³ and the
average diameter is greater than 1.2 μm, then a disc-stack centrifuge should be used for cell harvesting (Petrides, 1988). For dense and large microorganisms, centrifugation is more efficient for cell harvesting compared to membrane microfiltration. In this case, the microorganisms are large (average diameter 2-3 μm) and dense (average density 1100 Kg/m³) because of the inclusion bodies.

3. **What is the sublocation of product (Periplasmic or Cytoplasmic)?**
   If the product is in the periplasmic space, then the program also investigates the use of 'osmotic shock' for product release. In this case, however, the product is in the cytoplasm and cell disruption is assumed to break open the cells and release the inclusion bodies.

4. **Is feed to cell disruption less than 2 m³/h?** A bead mill is considered for cell disruption only if the feed to cell disruption process step is less than 2 m³/h. Bead mills of larger size are not available in the market. In this case, a high pressure homogenizer is assumed for cell disruption because the expected feed to cell disruption is higher than 2 m³/h.

5. **Recovery and washing of inclusion bodies.** Because inclusion bodies are fairly large (average diameter of 0.5-0.6 μm) and dense particles (density of 1.3 g/cm³), a disc-stack centrifuge is used for their recovery. Inclusion bodies are recovered in the heavy phase of the centrifuge while most of cell debris particles remain in the light phase. Prior to centrifugation, the broth is diluted to its initial volume to facilitate inclusion body separation from debris. This wash and separation step can be repeated more than once (twice in this case). The second wash
and dilution is done using a detergent solution (Triton-X-100) for more efficient purification.

6. *Inclusion body solubilization.* If the product is in the form of inclusion bodies, then unfolding and renaturation are required. The solubilization of inclusion bodies and unfolding of denatured pST is accomplished using a chaotropic agent, such as urea or guanidine hydrochloride, and a reducing agent, such as 2-mercaptoethanol or DTT. The process is carried out in a well mixed reactor.

7. *Buffer exchange.* Buffer exchange is required after solubilization to remove the chaotropic agent and create conditions appropriate for refolding. It is carried out either by ultrafiltration or by gel filtration. Ultrafiltration is more economical for large scale operation and was selected in this case.

8. *Protein refolding.* After buffer exchange, the solubilized protein is refolded back to its native form. The protein solution is diluted with water to a solids concentration of about 0.05% w/v. The dilution is necessary to ensure a minimum level of intermolecular interactions that lead to inactive product. The dilution water is saturated with oxygen to provide the required oxidative conditions for the reoxidation and refolding of pST molecules. The pH is controlled at 9.5 because that level facilitates reoxidation and refolding of pST (Snoswell, 1988). The process of reoxidation and refolding takes place in a well mixed reactor; the average residence time of the liquid in the reactor is 2 h.
9. Protein solution concentration. The dilute protein solution is concentrated using an ultrafilter of 10,000 MW cut-off.

10. Polishing. If the final product is required in high purity and chromatography units are used for final purification, then a polishing step is necessary to prevent particulate material from entering chromatography columns. Polishing is carried out by a dead-end filter that removes fine particles.

11. High resolution stages. If the final product is required in high purity, chromatography units are used. The first step is usually an ion exchange step that separates based on molecular charge. Ion exchange can handle large quantities of protein solution with significant purification and concentration. If an affinity chromatography resin is available and the product is required in very high purity (> 98%), then it may be used after ion exchange. The high resolution purification is usually completed by a gel filter that removes the small amounts of remaining impurities and also exchanges the buffer used for elution of the ion exchange and/or gel filtration chromatography columns.

The synthesized flowsheet, including the upstream section, is shown in Figure 8.1. DCF101 is the disc-stack centrifuge for cell harvesting. HMG101 is the high pressure homogenizer for cell disruption. DCF102 is a disc-stack centrifuge used for inclusion bodies recovery. The inclusion body paste is mixed with water and detergent using the M2X103 mixer. DCF103 carries out the second step of inclusion body recovery. The WMR101 well
mixed reactor is used for inclusion body solubilization. The UFL101 ultrafilter is used for buffer exchange. The WMR102 well mixed reactor is used for refolding. The UFL102 ultrafilter is used for protein solution concentration. The DEF101 dead-end filter is used for polishing. The INX101 ion exchanger is used for high resolution purification. Finally, purification is completed by the GFL101 gel filter unit.

8.4.2 Analysis of Downstream Section

The analysis of the downstream section also starts with the initialization of the flowsheet. The plant is assumed to operate 330 days/year with its upstream section working around the clock and its downstream section working 1 shift/day. The downstream section is oversized to minimize processing time and reduce product degradation.

Engineering specifications provided during initialization of downstream section unit operations include: **Cell Harvesting**: The v/v fraction of particles in the heavy phase is 40%. The viscosity of extracellular liquid is 1.3 cP. The efficiency factor (see Chapter 7 for definition) of the centrifuge is 30%. **Cell Disruption**: The broth is passed through the homogenizer 4 times at a pressure drop of 800 bar. This large number of passes and high pressure drop assure complete release of inclusion bodies and creation of small cell debris particles that facilitates their separation from inclusion bodies. **Inclusion Body Recovery**: The v/v fraction of particles in the heavy phase is 20%. The viscosity of extracellular liquid is 1.5 cP. The efficiency factor of the centrifuge is 30%. Triton-X-100 solution is used for washing between the two centrifugation steps to facilitate removal of impurities. **Inclusion Body Solubilization**: Solubilization is accomplished
with 6 M urea (chaotropic agent) and 0.5 M 2-mercaptoethanol (reducing agent that reduces disulfide bonds) solutions. The average residence time of the liquid in the well mixed reactor is 2 h. Solubilization of inclusion bodies is modeled as a stoichiometric reaction that yields unfolded pST and other contaminant proteins. **Buffer Exchange and Protein Solution Concentration:** The molecular weight cut-off of the ultrafilter membrane is 10,000. The average filtrate flux is 40 L/m²·h. **Ion Exchange:** Anion exchange is used because pST is negatively charged in the pH range in which it is stable. The type of resin is Fast Flow Mono Q (from Pharmacia). The binding capacity is 20 mg/ml and the cycle time is 2 h. It is assumed that the resin is replaced every 300 cycles and that the eluent volume per cycle is 2.5 times the column volume. **Gel Filtration:** The type of resin is Sephadex G25 or Sepharose CL (from Pharmacia). The sample volume is 10% of the column volume. The column length is 50 cm. The linear flowrate of the liquid through the column is 2 m/h. The overdesign factor is 1.2.

8.4.2.1 Material Balances

A detailed report of the material balances estimated by BioDesigner is given in Appendix IV. The final product has a purity of over 96% (g pST/g of total solids). The various component amounts are given in kg/Cycle (1 Cycle = 30 h).

Figure 8.2 shows the overall material balance of the entire plant per cycle. Note the small amount of product compared to feed through the whole plant and, as a consequence, the large amount of high organic waste water that is generated. Figure 8.3a shows the averaged amount of broth that flows through each unit operation. Note the high volume increase at
the refolding step. Finally, Figure 8.3b shows the cumulative yield after each unit operation. The overall recovery yield is about 24% (considering the amount of denatured pST in the inclusion bodies of the fermentation broth as the initial amount of product). The steps of cell disruption, solubilization, and refolding have the lowest yield. However, it is believed that by improving these steps an overall recovery yield of 50% can easily be achieved.

In the stream report (Appendix IV), the "Debris" component represents cell wall fragments and other components that have a particle size greater than 0.1 μm whereas the "Protein" component represents soluble cell components of low or high molecular weight. It is assumed that the inclusion bodies (Incl_Body) contain about 41% denatured pST (Den_pST) while the rest consists of other proteinaceous materials. The inclusion bodies are dissolved in the WMR101 reactor yielding denatured pST and protein. Denatured pST is converted to active pST in the WMR102 reactor. The sum of "Incl_Body", intracellular "Protein", and "Debris" in the S110 stream constitutes the total amount of dry cell mass formed in the fermentor.

8.4.2.2 Capital Cost Estimation

The cost estimation functions of BioDesigner are used to estimate the purchase cost of equipment. Table 8.2 shows the purchase cost of the major pieces of equipment of the flowsheet. Note that the fermentor and the compressors are the most expensive pieces of equipment in the upstream section. In the downstream section, the cost is more or less evenly
distributed among the centrifuges, the membrane filters, and the chromatography units.

Table 8.3 shows a summary of the fixed capital investment. The total fixed capital investment for a plant of this capacity (6,200 kg/yr) is about $47.3 million. The multipliers used in Table 8.3 were adapted and modified from projects for chemical processes. It is believed that their accuracy is within the range of preliminary design (±30%).

8.4.2.3 Operating Cost Estimation

A summary of the annual operating cost is presented in Table 8.4. Figure 8.4 shows graphically the percentage of each item. Note that the Direct Fixed Capital (DFC) dependent cost items dominate the annual operating cost (43.6%). The second most important item is the cost of process chemicals and other consumables (15.8%). Table 8.6 presents a breakdown of this cost. This item, besides the cost of actual chemicals, also includes the cost of filter membrane replacement as well as the cost of gel replacement for the chromatographic units.

The cost of labor-dependent items lies in the third position accounting for 14.7% of the annual operating cost. Table 8.7 shows the number of operators that are required per shift and per unit. The total number of operators per shift is 17. To estimate the number of operators, it was assumed that three shifts per day are used in the upstream section but only one in the downstream. The annual cost per operator was assumed to be $32,000. This does not include cost of fringe benefits.

The cost of R&D is the fourth most important item in the operating cost (13.5%). It was assumed that $3 million is spent on R&D annually. The cost
of Running Royalties was assumed equal to zero and the cost of Sales and Marketing was ignored. A breakdown of the Raw Materials cost is shown in Table 8.5. A breakdown of the Waste Treatment cost is shown in Table 8.8 while a breakdown of the Utilities cost is shown in Table 8.9.

**8.4.2.4 Profitability Analysis**

Table 8.10 shows a summary of the profitability analysis for the base case. The Total Investment for a plant of this capacity (6,200 kg/year) is about $49 million. The production cost of pST is $3,606/kg. For a selling price of $10,000/kg or $2/dose (1 dose = 200 mg), the project seems very profitable with a Gross Margin of 0.64, a Return On Investment of 64%, and a Payback Time of 1.57 years.

Table 8.11 shows the results of the Cash Flow Analysis. The Internal Rate of Return (IRR) before taxes is 0.37 and after taxes is 0.29. The Net Present Value (NPV) of the project is 140.9, 112.1, and 88.8 million dollars for interest of 8, 10, and 12% respectively. Table 8.12 shows a breakdown of capital outlay while Table 8.13 shows a breakdown of loan payment.

The following assumptions were made to carry out the Cash Flow Analysis: The project life is 18 years. The project is financed 60% by equity and 40% by debt. Plant construction starts in January of 1988 and lasts for 30 months. Production starts in July of 1990. The fixed capital investment is allocated 30% in the first year of construction, 40% in the second, and the remaining 30% in the third year. The salvage value of the plant (at the end of its life) is 5% of its initial value. The estimation of the working capital is based on a two-month period cost of labor-dependent items, raw materials, process chemicals, and utilities. A value of $15 million is assumed for up-
front R&D cost which is financed 80% by equity and 20% by debt. The loan for the direct fixed capital has an interest of 12% and a payment period of 10 years. The loan for the working capital and the up-front R&D has an interest of 15% and a payment period of 6 years. The direct fixed capital is depreciated using the "Sum of the Years Digits" method.

8.5 Sensitivity Analysis

BioDesigner is an efficient tool to answer "what if" questions and carry out sensitivity analysis. In this case study, the effect of recovery yield, utilization of downstream processing equipment, and plant capacity on the production cost of pST was examined.

8.5.1 Effect of Recovery Yield

In the base case, the overall product recovery yield was about 24% mainly due to low yield of homogenization, solubilization, and refolding (see Figure 8.3b). It is believed that the assumptions made for inclusion body release and product degradation during cell disruption were too pessimistic. It is also expected that further research in protein renaturation will result in significantly higher yield of solubilization and refolding in the future. Overall recovery yield of up to 60% is likely to be realized in the future. Figure 8.5 shows the effect of such likely recovery yield improvements on the production cost of pST.

8.5.2 Improving Equipment Utilization
In the base case, it was found that the DFC dependent cost dominates the production cost of pST (43.6% of the overall cost). Therefore, better utilization of the equipment may reduce the production cost. Such an opportunity exists because in the base case it was assumed that the downstream section is sized to process one batch (30 h) during one shift (8 h). To examine this opportunity, the production cost of pST was estimated as a function of the number of shifts/day in the downstream section (Fig. 8.6). As can be seen, by increasing the number of shifts/day to 2, a reduction of 14.5% is realized in the production cost of pST. However, a further increase to 3 shifts/day results in a slight increase in the production cost because the savings in equipment cost are less than the additional required labor cost. The optimum point, however, is also a function of plant capacity. It is expected that for larger plants the optimum value of shifts/day is 3.

8.5.3 Increasing Plant Capacity

To examine the effect of economy of scale, the production cost of pST was estimated as a function of plant capacity (Fig. 8.7). Note that the solid line in Figure 8.7 represents the cost of pST when 2 shifts/day are used in the downstream section. The base case (1 shift/day) is shown by the asterisk. As can be seen, significant savings of up to 28% (or up to 40% compared to base case) can be realized by increasing the plant capacity by approximately an order of magnitude.
Figure 8.1: Flowsheet of the production of Porcine Somatotropin
Figure 8.2: OVERALL MATERIAL BALANCE PER CYCLE (kg/30 h)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>2,100</td>
</tr>
<tr>
<td>Ammonia</td>
<td>136</td>
</tr>
<tr>
<td>Water</td>
<td>444,900</td>
</tr>
<tr>
<td>Salts</td>
<td>351</td>
</tr>
<tr>
<td>Detergent</td>
<td>33</td>
</tr>
<tr>
<td>Urea</td>
<td>2,130</td>
</tr>
<tr>
<td>MrEtOH</td>
<td>325</td>
</tr>
<tr>
<td>Oxygen</td>
<td>8,982</td>
</tr>
</tbody>
</table>

PROCESS UNITS:
- Batching
- Sterilization
- Fermentation
- Cell Harvesting
- Cell Disruption
- Inc_Body Recovery
- Solubilization
- Refolding
- Concentration
- Final Purification

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>pST</td>
<td>23</td>
</tr>
<tr>
<td>Organic Waste</td>
<td>3,642</td>
</tr>
<tr>
<td>CO2</td>
<td>1,504</td>
</tr>
<tr>
<td>Waste</td>
<td>444,731</td>
</tr>
<tr>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>7,935</td>
</tr>
</tbody>
</table>

\[ \Sigma \text{in} = \Sigma \text{out} = 458,957 \text{ kg/Cycle} \]
Figure 8.3: Schematic Presentation of the Material Balances
<table>
<thead>
<tr>
<th>Quantity/Stand-by</th>
<th>Description</th>
<th>Unit Cost ( $ )</th>
<th>Cost ( $ )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/0</td>
<td>BLT101 Blending Tank</td>
<td>37000</td>
<td>37000</td>
</tr>
<tr>
<td></td>
<td>Vol = 41.18 m^3, SS304, 8.8 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>STR101 Continuous Sterilizer</td>
<td>212000</td>
<td>212000</td>
</tr>
<tr>
<td></td>
<td>Capacity = 4.77 m^3/h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/1</td>
<td>CMP101 Centrifugal Compressor</td>
<td>243000</td>
<td>486000</td>
</tr>
<tr>
<td></td>
<td>DFress = 5.0 bar, SS316, 133.0 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>M2X101 Mixer</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>0.00 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>AFL101 Cartridge Air Filter</td>
<td>4000</td>
<td>4000</td>
</tr>
<tr>
<td></td>
<td>Air Inlet, SS316 Housing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>FRM101 Agitated Fermentor</td>
<td>282000</td>
<td>282000</td>
</tr>
<tr>
<td></td>
<td>Vol = 32.47 m^3, SS316, 73.1 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>FRM101 Agitated Seed Fermentor</td>
<td>75000</td>
<td>75000</td>
</tr>
<tr>
<td></td>
<td>Vol = 2.27 m^3, SS316, 5.1 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>BLT102 Blending Tank</td>
<td>36000</td>
<td>36000</td>
</tr>
<tr>
<td></td>
<td>Vol = 38.24 m^3, SS304, 8.1 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>DCF101 Disc-Stack Centrifuge</td>
<td>89000</td>
<td>89000</td>
</tr>
<tr>
<td></td>
<td>$\Sigma = 23187 \text{ m}^2, 12.2 \text{ kW}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>HMG101 High Pressure Homogenizer</td>
<td>30000</td>
<td>30000</td>
</tr>
<tr>
<td></td>
<td>Cap = 4607.7 L/h, 102.4 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>M2X102 Mixer</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>0.00 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/0</td>
<td>DCF102 Disc-Stack Centrifuge</td>
<td>281000</td>
<td>562000</td>
</tr>
<tr>
<td></td>
<td>$\Sigma = 193793 \text{ m}^2, 28.5 \text{ kW}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>M2X103 Mixer</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>0.00 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/0</td>
<td>DCF103 Disc-Stack Centrifuge</td>
<td>266000</td>
<td>532000</td>
</tr>
<tr>
<td></td>
<td>$\Sigma = 174718 \text{ m}^2, 27.4 \text{ kW}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>WMR101 Agitated Reactor</td>
<td>41000</td>
<td>41000</td>
</tr>
<tr>
<td></td>
<td>Vol = 2.46 m^3, SS316, 1.0 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>M2X104 Mixer</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>0.00 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/0</td>
<td>UFL101 Membrane Ultrafilter</td>
<td>72000</td>
<td>144000</td>
</tr>
<tr>
<td></td>
<td>Area = 76.9 m^2, 15.38 kW</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 8.2: (continued)

<table>
<thead>
<tr>
<th>Quantity/Stand-by</th>
<th>Description</th>
<th>Unit Cost ($)</th>
<th>Cost ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/0</td>
<td>WMR102 Agitated Reactor</td>
<td>240000</td>
<td>480000</td>
</tr>
<tr>
<td></td>
<td>Vol = 59.73 m$^3$, SS316, 25.4 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15/0</td>
<td>UFL102 Membrane Ultrafilter</td>
<td>73000</td>
<td>1095000</td>
</tr>
<tr>
<td></td>
<td>Area = 79.0 m$^2$, 15.79 kW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>DEF101 Dead-End Filter</td>
<td>26000</td>
<td>26000</td>
</tr>
<tr>
<td></td>
<td>Area = 22.5 m$^2$, PoreSize = 0.45 µm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/0</td>
<td>INX101 Ion Exchange Unit</td>
<td>223000</td>
<td>669000</td>
</tr>
<tr>
<td></td>
<td>L = 0.25 m, D = 0.86 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/0</td>
<td>GFL101 Gel Filtration Unit</td>
<td>213000</td>
<td>426000</td>
</tr>
<tr>
<td></td>
<td>L = 0.50 m, D = 0.72 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>AFL102 Cartridge Air Filter</td>
<td>10000</td>
<td>10000</td>
</tr>
<tr>
<td></td>
<td>Exhaust Gas, SS316 Housing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cost of Unlisted Equipment 20.0% of Total 1310000

**TOTAL EQUIPMENT PURCHASE COST** 6550000
### TABLE 8.3: FIXED CAPITAL ESTIMATE SUMMARY (1990 prices)

<table>
<thead>
<tr>
<th>A. TOTAL PLANT DIRECT COST (TPDC) (physical cost)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Equipment Purchase Cost (PC)</td>
</tr>
<tr>
<td>2. Installation (0.40 X PC)</td>
</tr>
<tr>
<td>3. Process Piping (0.35 X PC)</td>
</tr>
<tr>
<td>4. Instrumentation (0.60 X PC)</td>
</tr>
<tr>
<td>5. Insulation (0.03 X PC)</td>
</tr>
<tr>
<td>6. Electrical (0.15 X PC)</td>
</tr>
<tr>
<td>7. Buildings (0.75 X PC)</td>
</tr>
<tr>
<td>8. Yard Improvement (0.15 X PC)</td>
</tr>
<tr>
<td>9. Auxiliary Facilities (0.50 X PC)</td>
</tr>
<tr>
<td><strong>TPDC = 25739000</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. TOTAL PLANT INDIRECT COST (TPIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10. Engineering (0.25 X TPDC)</td>
</tr>
<tr>
<td>11. Construction (0.35 X TPDC)</td>
</tr>
<tr>
<td><strong>TPIC = 15444000</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. TOTAL PLANT COST (TPDC + TPIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12. Contractor's fee (0.05 X TPC)</td>
</tr>
<tr>
<td>13. Contingency (0.10 X TPC)</td>
</tr>
<tr>
<td><strong>Σ(12+13) = 6177000</strong></td>
</tr>
</tbody>
</table>

| D. DIRECT FIXED CAPITAL (DFC) TPC + 12 + 13       | 47360000  |
TABLE 8.4: ANNUAL OPERATING COST (1990 prices)

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. DFC-DEPENDENT ITEMS (DFC = $ 47360000)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depreciation</td>
<td>$</td>
<td>4499000</td>
</tr>
<tr>
<td>Maintenance Material (0.03 X DFC)</td>
<td></td>
<td>1421000</td>
</tr>
<tr>
<td>Insurance (0.01 X DFC)</td>
<td></td>
<td>474000</td>
</tr>
<tr>
<td>Local Taxes (0.02 X DFC)</td>
<td></td>
<td>947000</td>
</tr>
<tr>
<td>Factory Expense (0.05 X DFC)</td>
<td></td>
<td>2368000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9709000</td>
</tr>
<tr>
<td>2. LABOR-DEPENDENT ITEMS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Operating labor ( 17 X 32000 )</td>
<td></td>
<td>544000</td>
</tr>
<tr>
<td>b. Maintenance labor (0.03 X DFC)</td>
<td></td>
<td>1421000</td>
</tr>
<tr>
<td>c. Fringe benefits (0.40 X (a+b))</td>
<td></td>
<td>786000</td>
</tr>
<tr>
<td>d. Supervision (0.20 X (a+b))</td>
<td></td>
<td>393000</td>
</tr>
<tr>
<td>e. Operating supplies (0.10 X a)</td>
<td></td>
<td>54000</td>
</tr>
<tr>
<td>f. Laboratory (0.15 X a)</td>
<td></td>
<td>82000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3280000</td>
</tr>
<tr>
<td>3. ADMINISTRATION AND OVERHEAD EXPENSE (0.6 X (a+b+c))</td>
<td></td>
<td>1415000</td>
</tr>
<tr>
<td>4. RAW MATERIALS</td>
<td></td>
<td>501000</td>
</tr>
<tr>
<td>5. PROCESS CHEMICALS &amp; OTHER CONSUMABLES</td>
<td></td>
<td>3517000</td>
</tr>
<tr>
<td>6. UTILITIES</td>
<td></td>
<td>246000</td>
</tr>
<tr>
<td>7. WASTE TREATMENT</td>
<td></td>
<td>600000</td>
</tr>
<tr>
<td>8. RUNNING R&amp;D</td>
<td></td>
<td>3000000</td>
</tr>
<tr>
<td>9. RUNNING ROYALTIES</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>10. SALES COST</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TOTAL ANNUAL OPERATING COST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Including Depreciation</td>
<td></td>
<td>22268000</td>
</tr>
<tr>
<td>Excluding Depreciation</td>
<td></td>
<td>17769000</td>
</tr>
</tbody>
</table>
BREAKDOWN OF THE ANNUAL OPERATING COST

Running R&D
13.5%

Waste Treatment
2.7%

Utilities
1.1%

Process Chemicals
15.8%

Raw Materials
2.3%

Overhead
6.4%

Labor
14.7%

DFC-Dependent Cost
43.6%

Figure 8.4: Breakdown of the Annual Operating Cost
### TABLE 8.5: RAW MATERIALS (1990 prices)

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit Cost ($/kg)</th>
<th>Annual Amount (kg)</th>
<th>Cost ($/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>8.000e-04</td>
<td>117453600</td>
<td>94000</td>
</tr>
<tr>
<td>NH3</td>
<td>2.500e-01</td>
<td>35957</td>
<td>9000</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.500e-01</td>
<td>554400</td>
<td>249000</td>
</tr>
<tr>
<td>Salts</td>
<td>1.600e+00</td>
<td>92664</td>
<td>148000</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td><strong>501000</strong></td>
</tr>
</tbody>
</table>

### TABLE 8.6: PROCESS CHEMICALS & OTHER CONSUMABLES (1990 prices)

#### CHEMICALS

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit Cost ($/kg)</th>
<th>Annual Amount (kg)</th>
<th>Cost ($/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>5.000e-01</td>
<td>562320</td>
<td>281000</td>
</tr>
<tr>
<td>MrEtOH</td>
<td>8.000e+00</td>
<td>85774</td>
<td>686000</td>
</tr>
<tr>
<td>Detergent</td>
<td>7.000e+00</td>
<td>8712</td>
<td>61000</td>
</tr>
<tr>
<td>INX101_Eluent</td>
<td>0.02</td>
<td>3213062</td>
<td>64000</td>
</tr>
<tr>
<td>GFL101_Eluent</td>
<td>0.01</td>
<td>4019516</td>
<td>40000</td>
</tr>
<tr>
<td><strong>SUBTOTAL</strong></td>
<td></td>
<td></td>
<td><strong>1132000</strong></td>
</tr>
</tbody>
</table>

#### MEMBRANE or FILTER CLOTH

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Unit Cost ($/m²)</th>
<th>Annual Amount (m²)</th>
<th>Cost ($/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFL101</td>
<td>200.00</td>
<td>152</td>
<td>30000</td>
</tr>
<tr>
<td>UFL102</td>
<td>200.00</td>
<td>1173</td>
<td>235000</td>
</tr>
<tr>
<td>DEF101</td>
<td>180.00</td>
<td>1854</td>
<td>334000</td>
</tr>
<tr>
<td><strong>SUBTOTAL</strong></td>
<td></td>
<td></td>
<td><strong>599000</strong></td>
</tr>
</tbody>
</table>

#### CHROMATOGRAPHY RESINS

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Unit Cost ($/L)</th>
<th>Annual Amount (L)</th>
<th>Cost ($/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INX101</td>
<td>500.00</td>
<td>1428</td>
<td>714000</td>
</tr>
<tr>
<td>GFL101</td>
<td>200.00</td>
<td>5359</td>
<td>1072000</td>
</tr>
<tr>
<td><strong>SUBTOTAL</strong></td>
<td></td>
<td></td>
<td><strong>1786000</strong></td>
</tr>
</tbody>
</table>

**TOTAL** 3517000
TABLE 8.7: LABOR REQUIREMENT ESTIMATE SUMMARY

<table>
<thead>
<tr>
<th>Equipment</th>
<th>shifts/day</th>
<th># units</th>
<th># opers/shift</th>
<th># opers/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warehouse</td>
<td>1.0</td>
<td>1</td>
<td>1.00</td>
<td>1.0</td>
</tr>
<tr>
<td>BLT101</td>
<td>3.0</td>
<td>1</td>
<td>0.10</td>
<td>0.3</td>
</tr>
<tr>
<td>STR101</td>
<td>3.0</td>
<td>1</td>
<td>0.10</td>
<td>0.3</td>
</tr>
<tr>
<td>CMP101</td>
<td>3.0</td>
<td>1</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>M2X101</td>
<td>3.0</td>
<td>1</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>AFL101</td>
<td>3.0</td>
<td>1</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>FRM101</td>
<td>3.0</td>
<td>1</td>
<td>0.25</td>
<td>0.8</td>
</tr>
<tr>
<td>BLT102</td>
<td>3.0</td>
<td>1</td>
<td>0.10</td>
<td>0.3</td>
</tr>
<tr>
<td>DCF101</td>
<td>1.0</td>
<td>1</td>
<td>0.15</td>
<td>0.1</td>
</tr>
<tr>
<td>HMG101</td>
<td>1.0</td>
<td>1</td>
<td>0.10</td>
<td>0.1</td>
</tr>
<tr>
<td>M2X102</td>
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<td>1</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>DCF102</td>
<td>1.0</td>
<td>2</td>
<td>0.30</td>
<td>0.3</td>
</tr>
<tr>
<td>M2X103</td>
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<td>1</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>DCF103</td>
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<td>2</td>
<td>0.30</td>
<td>0.3</td>
</tr>
<tr>
<td>WMR101</td>
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<td>1</td>
<td>0.25</td>
<td>0.2</td>
</tr>
<tr>
<td>M2X104</td>
<td>1.0</td>
<td>1</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>UFL101</td>
<td>1.0</td>
<td>2</td>
<td>0.40</td>
<td>0.4</td>
</tr>
<tr>
<td>UFL102</td>
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<td>2</td>
<td>0.50</td>
<td>0.5</td>
</tr>
<tr>
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<td>15</td>
<td>3.00</td>
<td>3.0</td>
</tr>
<tr>
<td>DEF101</td>
<td>1.0</td>
<td>1</td>
<td>0.10</td>
<td>0.1</td>
</tr>
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<td>3</td>
<td>0.60</td>
<td>0.6</td>
</tr>
<tr>
<td>GFL101</td>
<td>1.0</td>
<td>2</td>
<td>0.40</td>
<td>0.4</td>
</tr>
<tr>
<td>AFL102</td>
<td>1.0</td>
<td>1</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>Packaging</td>
<td>1.0</td>
<td>1</td>
<td>1.00</td>
<td>1.0</td>
</tr>
<tr>
<td>Other</td>
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<td>1</td>
<td>1.00</td>
<td>2.0</td>
</tr>
</tbody>
</table>

SUBTOTAL: 11.7

TOTAL (Accounting for Weekends * 7/5): 16.4 ---+> 17

---

TABLE 8.8: WASTE TREATMENT (1990 prices)

<table>
<thead>
<tr>
<th>Type of Waste</th>
<th>Unit Cost ($/kg)</th>
<th>Annual Amount (Kg)</th>
<th>Cost ($/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sewer</td>
<td>2.000e-04</td>
<td>112182423</td>
<td>22000</td>
</tr>
<tr>
<td>Organic</td>
<td>6.600e-01</td>
<td>875633</td>
<td>578000</td>
</tr>
</tbody>
</table>

TOTAL: 600000
### TABLE 8.9: UTILITY REQUIREMENTS (1990 prices)

#### ELECTRICITY (7.0￠/KWH)

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Power (kW/Unit)</th>
<th>Annual Amount (kWh)</th>
<th>Cost ($/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLT101</td>
<td>8.8</td>
<td>11781</td>
<td>1000</td>
</tr>
<tr>
<td>CMP101</td>
<td>133.0</td>
<td>632237</td>
<td>44000</td>
</tr>
<tr>
<td>FRM101</td>
<td>78.2</td>
<td>371448</td>
<td>26000</td>
</tr>
<tr>
<td>BLT102</td>
<td>8.1</td>
<td>19305</td>
<td>1000</td>
</tr>
<tr>
<td>DCF101</td>
<td>12.2</td>
<td>24150</td>
<td>2000</td>
</tr>
<tr>
<td>HMG101</td>
<td>102.4</td>
<td>202738</td>
<td>14000</td>
</tr>
<tr>
<td>DCF102</td>
<td>28.5</td>
<td>112923</td>
<td>8000</td>
</tr>
<tr>
<td>DCF103</td>
<td>27.4</td>
<td>108338</td>
<td>8000</td>
</tr>
<tr>
<td>WMR101</td>
<td>1.0</td>
<td>2067</td>
<td>0</td>
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<tr>
<td>UFL101</td>
<td>15.4</td>
<td>60922</td>
<td>4000</td>
</tr>
<tr>
<td>WMR102</td>
<td>25.4</td>
<td>100526</td>
<td>7000</td>
</tr>
<tr>
<td>UFL102</td>
<td>15.8</td>
<td>469108</td>
<td>33000</td>
</tr>
<tr>
<td>Unlisted Equipment (5% of total)</td>
<td></td>
<td></td>
<td>9000</td>
</tr>
<tr>
<td>General Load (8% of total)</td>
<td></td>
<td></td>
<td>14000</td>
</tr>
<tr>
<td><strong>SUBTOTAL</strong></td>
<td></td>
<td></td>
<td><strong>170000</strong></td>
</tr>
</tbody>
</table>

#### CHILLING ($25.0/10^6 kcal)

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Duty (kcal/h/Unit)</th>
<th>Annual Amount ($10^6 kcal)</th>
<th>Cost ($/yr)</th>
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#### COOLING WATER (25 C $\rightarrow$ 35 C) ($0.04/1000 kg)

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<th>Cost ($/yr)</th>
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TABLE 8.9: (continued)

STEAM ($4.40/1000 kg)

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TABLE 8.10: PROFITABILITY ANALYSIS (1990 prices)

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<td>E. PRODUCTION COST ($/kg) PST</td>
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<td>G. REVENUE ($/year)</td>
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</tr>
<tr>
<td>H. ANNUAL OPERATING COST</td>
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<tr>
<td>I. GROSS PROFIT ( G - H )</td>
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</tr>
<tr>
<td>J. TAXES (33 %)</td>
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</tr>
<tr>
<td>K. NET PROFIT ( I - J + Depreciation )</td>
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<td>RETURN ON INVESTMENT</td>
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### TABLE 8.11: CASH FLOW ANALYSIS

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<th>SALES REVENUES</th>
<th>OPERATING COST</th>
<th>GROSS PROFIT</th>
<th>LOAN PAYMENT</th>
<th>DEPRECIATION</th>
<th>TAXABLE INCOME</th>
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Depreciation Method: Sum-of-the-Years-Digits

NOTE: The above amounts are given in thousands of US dollars
### TABLE 8.12: BREAKDOWN OF CAPITAL OUTLAY (US $)

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<th>YEAR</th>
<th>DIRECT FIXED CAPITAL</th>
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<th>UP FRONT ROYALTIES</th>
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DFC Salvage Factor = 0.050

### TABLE 8.13: BREAKDOWN OF LOAN PAYMENT (US $)

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<th>WORKING CAPITAL</th>
<th>UP FRONT R&amp;D</th>
<th>UP FRONT ROYALTIES</th>
<th>TOTAL</th>
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Figure 8.5: Production cost of pST as a function of product recovery yield.

Figure 8.6: Production cost of pST as a function of the number of Shifts/Day in the downstream processing section.
Figure 8.7: Production cost of pST as a function of plant capacity
Chapter 9

Conclusions and Recommendations

9.1 Conclusions

This thesis has yielded the following conclusions.

1. Systematic design methodologies can decompose the difficult problem of bioprocess design into a number of easier subproblems in a hierarchical manner. Such methodologies present a consistent, scientific approach to bioprocess design.

2. The use of Knowledge Based Expert System (KBES) techniques in bioprocess synthesis can drastically reduce the large number of alternative flowsheets by using domain-specific design knowledge.

3. One can use computer graphics to easily represent unit operations and material streams that compose bioprocess flowsheets. The user-friendly representation allows the user to quickly develop alternative flowsheets and then efficiently apply analytical tools to the entire process.

BioDesigner, a software design tool, is the computer implementation of the systematic design methodology that was formulated as a part of this project. BioDesigner combines synthetic with analytic design capabilities but the emphasis is on interactive analysis.

BioDesigner has been used to carry out a number of case studies, including production of β-galactosidase, porcine somatotropin, γ-interferon, polyhydroxybutyrate, and mevinolin. The results evaluated by professionals
and were found realistic. A number of people, including students from two graduate classes (biochemical separations and biochemical engineering) at MIT, have used BioDesigner. Its ease of use and ability to analyze a large number of alternative designs in a short time makes it well suited for educational use.

9.2 Recommendations for Future Work

The design methodology and its computer implementation, that were developed as a part of this thesis, have several limitations and opportunities exist for future work.

Improve Synthetic Capabilities. To enable BioDesigner to reason on and evaluate intermediate analysis results, an improved representation of its object world is required. The current synthetic and analytic components of BioDesigner use different representations for the same objects (e.g., unit operations, chemical components) and the "cross-talk" between the two is limited. The flow of information is basically unidirectional, from the synthetic to the analytic component. Further, it is cumbersome to invoke procedures that carry out analytic tasks from the synthetic component.

The situation can be improved by reimplementing the analytic component in an object-oriented language (e.g., C++) and using the same object representation in the two components of BioDesigner. This would enable the synthetic component to have access to all object property variables and methods and facilitate reasoning on them. This suggested architecture was not possible when the development of BioDesigner was
begun because at that time there were no Macintosh-compatible, object-oriented languages easily interfaced to Nexpert Object.

**Improve Scheduling Capabilities.** The production of most high value biochemicals is carried out by processes that operate in batch or semicontinuous mode. A process step is initiated when the previous one has been completed or has proceeded to some extent. Pieces of equipment are often used to carry out more than one task; the same disc-stack centrifuge, for example, is used both for cell harvesting and cell debris removal. These are problems that cannot be adequately handled by a process simulator that assumes averaged continuous operation of unit operations. Some scheduling capabilities are needed for accurate sizing of equipment. Scheduling capabilities are also important for accurate calculation of utility requirements and estimation of operating labor.
References

Chapter 1


Chapter 2


Chapter 3


Pharmacia, #1. "Scale up to process chromatography", Pharmacia Fine Chemicals.


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Chapter 4


Chapter 5


Chapter 7

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Chapter 8


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Vize, D.P. and J.R.E. Wells (1987). Spacer alterations which increase the expression of porcine growth hormone in E. coli, FEBS LETTERS Published by Elsevier Science Publishers B.V. (Biomedical Division). Volume 213, number 1, 155-158.


Chapter 9


APPENDIX I

BioDesigner
The User's Guide
APPENDIX I: BioDesigner - User's Guide

I.1 Getting Started

What You Need

You can run the analytic component of BioDesigner on a very modest system:

- Macintosh Plus, Macintosh SE, Macintosh II
- 800K disk drive
- System 6.0 or later
- 1 Mega Byte of Random Access Memory (RAM)

To run the synthetic component of BioDesigner, which involves the use of the Nexpert Dynamic Library, 2 Mega Bytes of RAM and a hard disk are required.

Installing BioDesigner

Although BioDesigner application can be run from a floppy disk, you will probably want to install it onto a hard disk. Figure I.1 shows a recommended disk layout.

To install BioDesigner:

1. Start at the Finder and create a new folder; name it BIODESIGNER

2. Copy the BioDesigner file and the BioHelpFiles folder to BIODESIGNER. Never change the name of the BioHelpFiles folder.

3. Copy folders with desired design cases to BIODESIGNER.
Figure I.1: Recommended Disk Layout for BioDesigner
If you also want to use the synthetic component of BioDesigner:

4. Copy the Bio_KB_Files folder to BIODESIGNER.

5. Copy the NDL and NHI files to the System Folder.

Overview of BioDesigner

BioDesigner is a software tool designed to enhance the effectiveness of scientists and engineers engaged in process synthesis and analysis.

The analytic component of BioDesigner is a tool for efficiently carrying out design and evaluation of individual unit operations as well as integrated biochemical processes. It is intended to be used at the early stages of process design and during scale-up. It can enable chemical and biochemical engineers to quickly carry out a conceptual design for a new project idea and evaluate it from an economic point of view. For a synthesized flowsheet, the analytic component of BioDesigner carries out material and energy balances, estimates the size and cost of equipment, and carries out a detailed economic evaluation.

The synthetic component of BioDesigner assists the user in formulation of a flowsheet for the production of a biological product. BioDesigner has been interfaced to Nexpert Object, an expert system shell that maintains and provides easy access to experiential design knowledge needed in flowsheet synthesis. Nexpert Object is used in its runtime library version and it is called from within BioDesigner as an external subroutine.

BioDesigner has a user friendly interface that improves the communication between the user and the computer and minimizes the learning period.
I.2 Developing a Design Case

In this chapter you will develop a simple but non-trivial flowsheet design case, called Beta-Gal, for the production of an intracellular enzyme in *E. coli*, called β-galactosidase. The following steps summarize the process of developing a design case with BioDesigner:

1. Create a flowsheet using the **Unit_Ops** menu to select the required unit operations and the cross cursor (by clicking the => palette item) to draw the streams and connect the units.

2. Initialize the flowsheet using the first four items of the **Tasks** menu: *Check Flowsheet, Initialize Components, Initialize Feed Streams,* and *Initialize Unit_Ops.*

3. Complete the analysis of the flowsheet using the remaining items of the **Tasks** menu: *Solve M&E Balances, Generate Stream Report, Get Cost Data, Perform Economic Analysis.*

4. Save a design case by selecting *Save Design Case* under the **Design** menu.

Creating a Flowsheet

To create a process flowsheet with multiple unit operations, start with the **Unit_Ops** menu. To place a unit on the screen, you select a unit by highlighting its name and releasing the button (Figure I.2 shows how to select a stirred fermentor). Once on the screen, a unit can be moved around with hand cursor, can be flipped horizontally with the man cursor, and can be erased with the eraser cursor. BioDesigner uses a default naming system for identifying the unit operations. You can change the default name by first clicking the text palette item (T) to get the beam cursor (I) and then clicking the unit whose name you wish to change. This will display a
Figure I.2: Selection of a Fermentor from the Unit_Ops Menu
dialog window with the current name and purpose of the unit and will let you edit them.

Each unit operation has ports for inlet and outlet streams (see Figure I.3) for connection with other unit operations or feed and waste streams. There are three types of streams: feed streams, intermediate streams, and product streams (see Figure I.4). Feed streams do not have a source unit operation. Intermediate streams connect two unit operations. Product streams do not have a destination unit operation. Note that even waste streams are called product streams. All streams are automatically identified with a stream number.

To draw a stream you start by clicking the (=>) palette item to get the cross (+) cursor and then if the stream is a:

1. **Feed Stream.** You click anywhere on the open screen to initiate drawing of the stream and then click on the appropriate inlet port of the destination unit operation to terminate the stream. Between initiation and termination of the feed stream, the mouse may be clicked at intermediate points to create right angle bends; this permits customization of the stream route and flexibility in flowsheet design. BioDesigner automatically draws the feed stream symbol and identifies the stream.

2. **Intermediate Stream.** You click on the appropriate outlet port of the source unit operation and then on the appropriate inlet port of the destination unit operation to terminate the stream. You also can create specific routing by clicking the mouse wherever a right angle bend is desired. A stream can have up to twenty such bends.

3. **Product Stream.** You click on the appropriate outlet port of the source unit operation and then you double-click somewhere to terminate the stream line. Note that BioDesigner automatically draws the product stream symbol.

BioDesigner uses default names for each stream. To change these names you can use the "lens cursor" and click on a stream. A dialog window with some information about the stream appears and permits you
Figure I.3: Inlet and Outlet Stream Ports of a Disc-Stack Centrifuge

Figure I.4: Types of Material Streams
to edit the stream name and several other stream variables. You can move
the position of the stream name using the hand cursor.

**Flowsheet Description**

Figure I.5 shows an entire flowsheet for β-galactosidase production. It
was created using the "Save PICT As..." item of the File menu of
BioDesigner and edited and printed using MacDraw. The conceptual
design of the plant in this example is based on a production of purified β-
galactosidase of about 7,000 Kg/year. The upstream section is sized to
operate around the clock while the downstream section processes one batch
during one shift.

Fermentation media are prepared in the stainless steel tank (BLT101)
and are sterilized by a continuous sterilizer (STR101). A centrifugal
compressor (CMP101) and an absolute air filter (AirL101) provide sterile air
to fermentors at a rate of 1 VVM and at a discharge pressure of 6 barg.
Gaseous ammonia (S105), which is used as nitrogen source, is mixed with
the air inlet stream (S107). The fermentation time and downtime in the
production fermentor are 18 and 12 h, respectively, resulting in a cycle time
of 30 h.

The first step of the downstream section is cell harvesting to reduce the
volume of the broth and get rid of extracellular impurities; it is carried out
by a disc-stack centrifuge. Since β-galactosidase is an intracellular product,
the next step is cell disruption and is carried out by a high pressure
homogenizer. Another disc-stack centrifuge (DCF102) is used to remove
most of the cell debris particles. The remaining cell debris particles are
removed by a dead-end polishing filter (DEF101). The protein solution is
concentrated by an ultrafilter (UFL101). The final purification is achieved
by an ion exchange (INX101) and a gel filter unit (GFL101).

**Flowsheet Initialization**
Figure 1.5: Flowsheet for the production of beta-galactosidase
In order to complete material and energy balances and estimate the size of equipment, it is necessary to initialize the flowsheet with information on feed streams and unit operation performance data. Flowsheet initialization is done under the **Tasks** menu.

**Check Flowsheet**

It checks a flowsheet to be sure that all streams are drawn, whether any two streams have the same source or destination port, and it detects recycle loops and selects tear streams. Then problems are located and correcting actions are requested.

**Initialize Components...**

It displays the dialog window (Figure I.6) and permits you to specify the chemical components and their properties that are used in the simulation of the flowsheet. Default values are provided for some frequently used components. Their names and properties are easily changed. The global component properties in the current version of BioDesigner include:

- Molecular Weight
- Particle Size [μm]
- Density [kg/m³]

The values of other properties needed by some unit operations are provided during unit operation initialization. You specify the name of the microorganism (if one is used) and its properties (including the water content). Finally, you may also specify an activity basis for the product in Units of activity per gram of pure product. This is used to estimate the activity of the various streams during the material balances. Note that you can specify any number of chemical components. The components selected for the Beta-Gal design case include: b-Gal (the product), H₂O, CO₂, Debris, Protein (represents all contaminant proteins), Glucose (carbon source), NH₃ (nitrogen source), O₂, N₂, and Salts (media salts).

**Initialize Feed Streams...**

This menu item displays the dialog window shown in Figure I.7 once for each Feed stream of a flowsheet. You specify the flowrates of the various
Figure I.6: Dialog Window to Specify Chemical Components

Figure I.7: Dialog Window to Display and Let you Edit Information for a Stream
components as well as their intracellular and extracellular fractions. You also specify the stream temperature and pressure. Note that you do not have to specify the stream activity because it is estimated by the system.

**Initialize Unit_Ops...**

This menu item displays different dialog windows for the various unit operations and lets you provide engineering specifications. For instance, Figure I.8 displays the dialog window used for the initialization of a disc-stack centrifuge. The user provides values for the variables of the upper portion of the window (above the dotted line). However, for most of those variables there are default values that can be used during a first pass until better values are available. The values of the variables in the lower portion of the window (underneath the dotted line) are normally estimated by the system. However, if you want to provide your own value for a specific variable, you can do that by typing the value and then checking the corresponding check box. If you do so, the value that you type overrides the one that is estimated by the system.

**Flowsheet Analysis and Evaluation**

To analyze and evaluate a flowsheet, you use the next four items of the **Tasks** menu.

**Solve M&E Balances**

This carries out the material and energy balances and estimates the size and purchase cost of equipment. Next, you can use the lens cursor to look at the design results by simply clicking on a unit or stream.

**Generate Stream Report...**

It creates a file and prints out a detailed stream report in TEXT format. It also displays a dialog window and lets you select the format of the stream report. Table I.1 shows a part of the stream report for the Beta-Gal design case. For each stream, BioDesigner prints out the names of the source and destination unit operations, some stream properties, the
Figure I.8: Dialog Window to Display and Let you Edit Information of a Disc-Stack Centrifuge.

Figure I.9: Dialog Window to Specify the Unit Cost of Raw Materials and Process Chemicals and the Value of Product(s).
flowrates of intracellular components, the flowrates of extracellular components, the subtotal flowrates, and the total flowrate.

**Get Cost Data...**

It displays three dialog windows and lets you provide data needed for the economic evaluation. Figure I.9 displays the third window which specifies whether components are raw materials or process chemicals; which components constitute organic waste; and to specify the unit cost of raw materials and process chemicals and the selling price of product(s).

**Perform Economic Analysis...**

It creates a file and prints out a detailed economic analysis report. Table I.2 is the first Table of the economic analysis report for the β-galactosidase example. It shows the purchase cost of each unit operation along with a description of the unit.
### Table 1.1: PORTION OF THE STREAM REPORT OF THE Beta-Gal DESIGN CASE

<table>
<thead>
<tr>
<th>STREAM NAME</th>
<th>S111</th>
<th>S112</th>
<th>S113</th>
<th>S114</th>
<th>S115</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOURCE</td>
<td>AFL102</td>
<td>FRM101</td>
<td>FEED</td>
<td>BLT102</td>
<td>DCF101</td>
</tr>
<tr>
<td>DESTINATION</td>
<td>PRODUCT</td>
<td>BLT102</td>
<td>BLT102</td>
<td>DCF101</td>
<td>HMGI01</td>
</tr>
</tbody>
</table>

#### STREAM PROPERTIES

<table>
<thead>
<tr>
<th>ACTIVITY</th>
<th>U/ml</th>
<th>TEMP</th>
<th>C</th>
<th>PRES</th>
<th>BAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>38.8</td>
<td>25.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

#### INTRACELLULAR COMPONENTS FLOWRATE (kg/Cycle)

<table>
<thead>
<tr>
<th>Component</th>
<th>b-Gal</th>
<th>Debris</th>
<th>H2O</th>
<th>CO2</th>
<th>Glucose</th>
<th>NH3</th>
<th>O2</th>
<th>N2</th>
<th>Salts</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowrate</td>
<td>0.000</td>
<td>89.180</td>
<td>0.000</td>
<td>89.180</td>
<td>87.396</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

#### EXTRACELLULAR COMPONENTS FLOWRATE (kg/Cycle)

<table>
<thead>
<tr>
<th>Component</th>
<th>b-Gal</th>
<th>Debris</th>
<th>H2O</th>
<th>CO2</th>
<th>Glucose</th>
<th>NH3</th>
<th>O2</th>
<th>N2</th>
<th>Salts</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowrate</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**SUBTOTAL**

<table>
<thead>
<tr>
<th>Component</th>
<th>b-Gal</th>
<th>Debris</th>
<th>H2O</th>
<th>CO2</th>
<th>Glucose</th>
<th>NH3</th>
<th>O2</th>
<th>N2</th>
<th>Salts</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowrate</td>
<td>0.000</td>
<td>2924.647</td>
<td>0.000</td>
<td>2924.647</td>
<td>2866.154</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**SUBTOTAL**

<table>
<thead>
<tr>
<th>Component</th>
<th>b-Gal</th>
<th>Debris</th>
<th>H2O</th>
<th>CO2</th>
<th>Glucose</th>
<th>NH3</th>
<th>O2</th>
<th>N2</th>
<th>Salts</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowrate</td>
<td>39017.166</td>
<td>21095.387</td>
<td>0.000</td>
<td>21095.387</td>
<td>3313.372</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**TOTAL**

<table>
<thead>
<tr>
<th>Component</th>
<th>b-Gal</th>
<th>Debris</th>
<th>H2O</th>
<th>CO2</th>
<th>Glucose</th>
<th>NH3</th>
<th>O2</th>
<th>N2</th>
<th>Salts</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowrate</td>
<td>39017.166</td>
<td>24020.034</td>
<td>0.000</td>
<td>24020.034</td>
<td>6179.526</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

-188-
<table>
<thead>
<tr>
<th>Quantity/Stand-by</th>
<th>Description</th>
<th>Unit Cost</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/0</td>
<td>BLT101 Blending Tank</td>
<td>34000</td>
<td>34000</td>
</tr>
<tr>
<td></td>
<td>Vol = 35.29 m³, SS304, 7.5 kw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/0</td>
<td>STR101 Continuous Sterilizer</td>
<td>212000</td>
<td>106000</td>
</tr>
<tr>
<td></td>
<td>Capacity = 4.78 m³/h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>CMP101 Centrifugal Compressor</td>
<td>312000</td>
<td>312000</td>
</tr>
<tr>
<td></td>
<td>DPress = 5.0 bar, SS316, 174.4 kw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>AFL101 Cartridge Air Filter</td>
<td>4000</td>
<td>4000</td>
</tr>
<tr>
<td></td>
<td>Air Inlet, SS316 Housing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>FRM101 Agitated Fermentor</td>
<td>282000</td>
<td>282000</td>
</tr>
<tr>
<td></td>
<td>Vol = 32.47 m³, SS316, 73.0 kw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>FRM101 Agitated Seed Fermentor</td>
<td>75000</td>
<td>75000</td>
</tr>
<tr>
<td></td>
<td>Vol = 2.27 m³, SS316, 5.1 kw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>AFL102 Cartridge Air Filter</td>
<td>10000</td>
<td>10000</td>
</tr>
<tr>
<td></td>
<td>Exhaust Gas, SS316 Housing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>BLT102 Blending Tank</td>
<td>36000</td>
<td>36000</td>
</tr>
<tr>
<td></td>
<td>Vol = 38.82 m³, SS304, 8.2 kw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>DCF101 Disc-Stack Centrifuge</td>
<td>119000</td>
<td>119000</td>
</tr>
<tr>
<td></td>
<td>Σ = 39236 m², 15.1 kw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>HMG101 High Pressure Homogenizer</td>
<td>31000</td>
<td>31000</td>
</tr>
<tr>
<td></td>
<td>Cap = 5049.1 L/h, 112.2 kw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/0</td>
<td>DCF102 Disc-Stack Centrifuge</td>
<td>250000</td>
<td>500000</td>
</tr>
<tr>
<td></td>
<td>Σ = 155432 m², 26.1 kw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>DEF101 Dead-End Filter</td>
<td>11000</td>
<td>11000</td>
</tr>
<tr>
<td></td>
<td>Area = 6.1 m², PoreSize = 0.45 μm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>UFL101 Membrane Ultrafilter</td>
<td>45000</td>
<td>45000</td>
</tr>
<tr>
<td></td>
<td>Area = 30.7 m², 6.10 kw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/0</td>
<td>INX101 Ion Exchange Unit</td>
<td>233000</td>
<td>1165000</td>
</tr>
<tr>
<td></td>
<td>L = 0.25 m, D = 0.94 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>GFL101 Gel Filtration Unit</td>
<td>159000</td>
<td>159000</td>
</tr>
<tr>
<td></td>
<td>L = 0.50 m, D = 0.95 m</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cost of Unlisted Equipment
20.0% of Total

TOTAL EQUIPMENT PURCHASE COST

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I.3 Initialization of Unit Operations

To initialize a unit operation, you must provide values for some design variables. However, for most of the design variables there are good default values that can be used during a first pass.

All unit operations have some common variables located in the lower portion of the dialog windows whose values are normally estimated by the system. These variables include: Number of Units, Unit Cost, Year, Power, Heating Duty, Cooling Duty, etc. If the user, however, types his/her own value and checks the corresponding check box, then that value will override the one that is estimated by the system.

Utilization Factor

Most unit operations have a variable called utilization factor. The meaning of that variable is the following:

If $t_c$ is the cycle time of a fermentation plant (e.g., 40 h) and $t_u$ is the time that a unit operation is on (functioning) during that cycle time (e.g., 12 h), then the utilization factor of the unit is:

$$
\text{Utilization Factor} = \frac{t_u}{t_c} = \frac{\text{(Time Unit is On)}}{\text{(Cycle Time)}}
$$

The Utilization Factor of the various unit operations of a flowsheet is taken into account in the size estimation of equipment and the estimation of utilities consumption.

Unit Operation Specific Information

Use Chapter 7 of this thesis as a guide for the mathematical models used to describe the various unit operations of the current version of
BioDesigner. It is easier to understand why certain information is required for initialization if you are familiar with the model in which it is used.

**Fermentor**

1st Dialog Window. To initialize a fermentor you specify its liquid volume or the fermentation time. For continuous operation, the fermentation time is equal to the residence time of the broth in the fermentor. There are default values, which you can change, for the following variables: (Liquid Vol)/(Total Vol), (Height)/(Diameter), Downtime, Agitation Rate. You also have to specify the fermentation temperature because it is needed for the estimation of the cooling duty.

2nd Dialog Window. The fermentor model of the current version of BioDesigner is a stoichiometric reactor. The user provides the extent of reaction and the overall stoichiometry and the system carries out the material balances. Note that mass and not mole stoichiometric coefficients are required. The user provides an overall reaction of the following form:

\[
\text{Glucose} + \text{NH}_3 + \text{O}_2 \rightarrow \text{Product} + \text{Biomass} + \text{H}_2\text{O} + \text{CO}_2
\]

<table>
<thead>
<tr>
<th>Kg</th>
<th>-1.0</th>
<th>-0.1</th>
<th>-0.4</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.3</th>
</tr>
</thead>
</table>

The sum of all coefficients must be equal to zero (reactants have negative signs). The extent of reaction refers to the limiting component. The user also has to specify which components are volatile (exit in the gas outlet) and which constitute part of dry cell weight. Finally, the user has to specify the location of products (intracellular or extracellular).

3rd Dialog Window. As you may have noticed in the examples, seed and inoculum fermentors are not shown on flowsheets. Their cost, however, is taken into account and the initialization is done through this dialog window. The user specifies the volume of the seed fermentor(s) as a percentage of the production fermentor(s), the number of seed fermentors required per production fermentor, etc.

**Other Fermentors and Reactors**
The other bioreactor models are very similar to stirred fermentor in terms of initialization.
Blending Tank

This unit operation has only one initialization dialog window which looks like a simplified version of the fermentor's first window.

Disc-Stack Centrifuge

Select Design Mode and specify the design component if you wish the system to estimate the size of the centrifuge. Design component is the particle component that is desired to be recovered in the heavy phase of the centrifuge. Select Rate Mode and specify the Sigma factor of the centrifuge if you have a centrifuge of given size and wish the system to estimate the performance. The Design Component Recovery Yield is taken into account only when you select Design Mode.

High Pressure Homogenizer

Modify the kinetic constants, if you have values better than the default ones. Specify which intracellular components are released by typing True or just t in the second column of the table on the lower left side.

Membrane Microfilter

1st Dialog Window. Select Design Mode if you know the desired Concentration Factor and want the system to estimate the size of the unit. Check all default values of the various design variables.

2nd Dialog Window. Provide the Rejection Coefficient of the various components (1 for 100% rejection, 0 for no rejection). If you want to simulate degradation of a product in a microfilter, provide the name of the component that denatures Active Product, the name of the denatured form of the product, and the denaturation fraction (0.05 for 5% denaturation).

Membrane Ultrafilter

Very similar to microfilter. The only difference is in the Molecular Weight Cut Off instead of the Membrane Pore Size.

Rotary Vacuum Filter (RV_Filtter)

1st Dialog Window. Check the default values for Cake Porosity and Filtrate Flux.
2nd Dialog Window. Provide the Retention Coefficient for the various components (1 for full retention, 0 for no retention).

Gel Filtration

1st Dialog Window. Check the various default values. Specify appropriate values for Purification Factor and Recovery Yield that make sense in your case.

2nd Dialog Window. Check value of Gel Replacement Frequency (number of cycles) that shows after how many cycles the gel is replaced.

Ion Exchanger

1st Dialog Window. Check the various default values. Specify appropriate values for Purification Factor and Recovery Yield that make sense in your case.

2nd Dialog Window. Provide values for the Binding Fraction (1 for full binding on the resin, 0 for no binding) of the various components.

Affinity Chromatography

Almost identical to ion exchange.

Other Unit Operations

The initialization of most other unit operations is fairly obvious to the user.
I.4 Synthetic Capabilities of BioDesigner

BioDesigner has been interfaced to Nexpert Object, an expert system shell that can process experiential design knowledge to carry out synthesis. Nexpert Object is used in its runtime library version and it is called from within BioDesigner as an external subroutine.

Figure I.10 shows the interface of BioDesigner to Nexpert Object. During the synthesis of the upstream or downstream section, the user goes through a dialog with the system. The system asks questions about the properties of the product, the properties of the producing system, the properties of the contaminants, etc. At the end, it comes up with a reasonable flowsheet for the production of the desired product.

To use the synthetic part of BioDesigner you must have a hardware key for the Nexpert Dynamic Library or for the Development version of Nexpert Object (Nexpert Object is a copy-protected commercial product marketed by Neuron Data, Inc.). If you do not have the key, never select Synthesize Upstream or Synthesize Downstream because the current version of BioDesigner will crash.
Figure I.10: Interface to Synthetic Part of BioDesigner
I. 5 Auxiliary Facilities

Quick Elemental Balance

BioDesigner allows the user to quickly estimate the amount and cost of raw materials required for the production of a certain amount of product. This is done by the facilities provided through the Input-Output Analysis hierarchical menu (see Figure I.11). More specifically, through Elemental Balance... the user can quickly do an elemental balance to estimate the stoichiometry of the overall macroscopic reaction taking place in a bioreactor (see Figure I.12). Through Recovery Yield..., one can quickly estimate the overall product recovery yield based on the properties of the product and the producing system. Finally, through Economic Potential..., one can quickly estimate the cost of raw materials.

Figure I.13 shows the dialog window used to make a first estimate of the overall recovery yield in the downstream section. By selecting a category of products, the system displays the range of expected recovery yield and a selected average. The value of the selected average can be edited by the user.

Based on the elemental balance and the molecular weight of the various components, the system estimates the overall material balance (see 2nd column of the Table in Figure I.14). The user provides the unit price of product and then by clicking Calculate the system estimates the Economic Potential as defined in the upper portion of the same figure. The value of the overall recovery yield is also taken into account in the estimation of the economic potential.
Figure I.11: Menu of Auxiliary Facilities

Figure I.12: Elemental Balance Facility
Figure I.13: Recovery Yield Estimation Facility

Figure I.14: Economic Potential Estimation Facility
Scheduling Facility

BioDesigner has some scheduling capabilities to handle unit operations that operate in batch or semicontinuous mode. To generate a schedule for the current design case, choose Generate Schedule... from the Tasks menu. The dialog window that is shown on the top of Figure I.15 will appear and will let you specify the process time, turn-around time, and starting time of each unit operation. Clicking OK will store the data with the flowsheet and dismiss the dialog box, Cancel will dismiss the dialog box without saving the changes to the data, Create Chart will save the data, dismiss the dialog box, and draw a scheduling chart (graph on the bottom of Figure I.15).

As you can see, the dialog box has several data fields. The Cycle Time field represents the time for one complete production run of the entire process. Each unit has three time values associated with it. The process time is the amount of time the unit actually runs. The turn around time is the time required to prepare the unit for the next run, and the starting time is how long the unit will wait from the beginning of the cycle before running. All cycles begin at time 0.0. Note that the end of a unit’s activity comes at: \( T_{\text{start}} + T_{\text{process}} + T_{\text{turn-around}} \), which must be less than or equal to \( T_{\text{cycle}} \).

I5.3 Help Facility

BioDesigner's Help facility (Figure I.16) provides information on "How to use BioDesigner", provides information about the various "Unit
Figure 1.15: The Dialog Window on the Top is used to provide Scheduling Information. The Schedule Chart on the bottom shows the operation time (dark section of a bar) and preparation for the next batch time (gray section of a bar) of a unit operation.
Operations", about "Cell Composition" of various microorganisms, about "Media Preparation", and "Raw Material Prices".

The Help facility that provides information about cell composition, media preparation, and raw material prices is editable to allow the user to easily provide and document his/her own knowledge.

The dialog window on the top of Figure I.16 is the help facility that provides information on how to use BioDesigner. The user selects a topic from the list (small window on the right) and the system displays relevant information in the window on the left (see dialog window on the bottom of Figure I.16). The help facility can display both text and graphics.
Welcome to BioDesigner Help Facility

Select an Item in the List to get Information about it!

Credit to Joe Pillera for Initial Source Code

Figure I.16: Dialog Windows of the Help Facility
I.6 BioDesigner's Menus

File Menu

[Menu: File]

- **New Text**: Creates and opens a TEXT document.
- **Open Text...**: Opens a TEXT file that has been created previously.
- **Close...**: Closes the current active window (TEXT or FLOWSHEET window).
- **Save**: Writes to the TEXT file any changes you have made since you last opened or saved the file.
- **Save PICT As...**: Saves a flowsheet in PICT format. The picture of a flowsheet saved in PICT format can be opened by other graphics programs, such as MacDraw, for further modification. This menu item changes to 'Save TEXT As...' when a TEXT window is the active window.
- **Print...**: 

---

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Prints a TEXT or a Flowsheet document.

Quit BioDesigner®
Exits BioDesigner and returns to Finder.

Edit Menu

<table>
<thead>
<tr>
<th>Edit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undo</td>
</tr>
<tr>
<td>Cut</td>
</tr>
<tr>
<td>Copy</td>
</tr>
<tr>
<td>Paste</td>
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<tr>
<td>Clear</td>
</tr>
<tr>
<td>Refresh Screen</td>
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<tr>
<td>Review\Change</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Drawing Size...</th>
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<tbody>
<tr>
<td>Text Styles...</td>
</tr>
<tr>
<td>Page Breaks</td>
</tr>
<tr>
<td>Reduced View</td>
</tr>
<tr>
<td>Axis Tick Marks</td>
</tr>
<tr>
<td>Default Settings</td>
</tr>
<tr>
<td>Preferences</td>
</tr>
</tbody>
</table>

Undo
This is not used in the current version of BioDesigner.

Cut
Removes the selected text item and puts it on the Clipboard.

Copy
Covers the selected text item to the Clipboard.

**Paste**
Pastes a text item from the Clipboard.

**Clear**
Deletes the currently selected text item without copying it to the Clipboard.

**Refresh Screen**
Redraws everything on the active flowsheet window.

**Drawing Size...**
Displays and lets you change information about the size of a flowsheet (# of pages).

**Text Styles...**
Displays and lets you change information about the size and font of text.

**Page Breaks...**
Draws page break lines.

**Reduced View**
Zoom out facility.

**Axis Tick Marks...**
Lets you change tick marks interval on the x-axis of schedule graphs.

**Default Settings...**
Lets you change the default settings for the various types of windows that BioDesigner handles.

**Preferences...**
Lets you change the format of stream dialog windows, the cycle time of the entire process, etc.
Review\Change Hierarchical Menu

Stand-by Units...
Displays and lets you change information about the number of stand-by units for each unit operation of a flowsheet.

Fixed Capital Cost Multipliers...
Displays and lets you change information about the multipliers used for the estimation of the Fixed Capital Investment of a design case.

Annual Operating Cost Multipliers...
Displays and lets you change information about the multipliers used for the estimation of the Annual Operating Cost of a design case.

<table>
<thead>
<tr>
<th>Edit</th>
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<tbody>
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<td>Undo ※Z</td>
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<td>Cut ※K</td>
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<td>Copy ※C</td>
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<tr>
<td>Paste ※V</td>
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<tr>
<th>Review Change</th>
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<tr>
<td>Drawing Size...</td>
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<td>Reduced View</td>
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<tr>
<td>Rulers Tick Marks...</td>
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<tr>
<td>Default Settings...</td>
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<tr>
<th>Stand-by Units...</th>
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<tbody>
<tr>
<td>Fixed Capital Cost Multipliers...</td>
</tr>
<tr>
<td>Annual Operating Cost Multipliers...</td>
</tr>
<tr>
<td>Labor Requirement Factors...</td>
</tr>
<tr>
<td>Utility Unit Cost...</td>
</tr>
<tr>
<td>Unit Op Utility Efficiency...</td>
</tr>
<tr>
<td>Economic Parameters...</td>
</tr>
</tbody>
</table>

Preferences ※P
Labor Requirement Factors...
Displays and lets you change information about the factors used for the estimation of Labor requirement.

Utilities Unit Cost...
Displays and lets you change information about the unit cost of utilities.

Unit_Op Utility Efficiency...
Lets you specify the efficiency for electricity, heating, and cooling that a unit operation consumes.

Economic Parameters...
Displays and lets you change information that is used in Economic Evaluation (method of Depreciation, Capital Outlay profile, etc.).

Design Menu

Input-Output Analysis
A hierarchical menu used to carry out the input-output analysis (see next section).

Design Menu

<table>
<thead>
<tr>
<th>Input-Output Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesize Upstream...</td>
</tr>
<tr>
<td>Synthesize Downstream...</td>
</tr>
<tr>
<td>Load Design Case...</td>
</tr>
<tr>
<td>Save Design Case...</td>
</tr>
<tr>
<td>Clear Design Case...</td>
</tr>
</tbody>
</table>

Synthesize Upstream...
Loads the Nexpert Dynamic Library (NDL) and initiates the synthesis of the upstream section of a flowsheet.
**Synthesize Downstream...**
Initiates the synthesis of the downstream section of a flowsheet.

**Load Design Case...**
Lets you load a design case from a disk.

**Save Design Case...**
Lets you save the current design case on a disk.

**Clear Design Case...**
Lets you remove the current design case from the computer memory.

**Input-Output Analysis Hierarchical Menu**

**Elemental Balance...**
Lets you easily carry out an elemental balance around a reaction system.

**Recovery Yield...**
Displays and lets you change information about the overall recovery yield used in the Input-Output Analysis.
Lets you estimate the economic potential of a project at the first level of design.

**Tasks Menu**

**Check Flowsheet**
Checks a flowsheet for any inconsistencies.

**Initialize Components...**
Lets you specify the chemical components and their properties that are used in a flowsheet.

**Initialize Feed Streams...**
Lets you initialize the feed streams of a flowsheet. You provide the flowrates of the various components and some stream properties for each feed stream.

**Initialize Unit_Ops...**
Lets you initialize the various unit operations of a flowsheet.
Solve M&E Balances
This is used after the completion of the initialization of a flowsheet to calculate the material and energy balances and estimate the size and purchase cost of equipment.

Generate Stream Report...
Creates a file and generates a detailed stream report. The file format is simple TEXT (ASCII file).

Get Cost Data...
Displays and lets you change information required for the economic evaluation.

Perform Economic Analysis...
Creates a TEXT file and generates a detailed economic analysis report.

Do Complete Analysis...
Combines the estimation of the material and energy balances with the economic evaluation.

Schedule...
Lets you specify the starting, process, and turn-around times for the various unit operations of a flowsheet and then creates a scheduling bar chart that shows when a unit is functioning.

Unit_Ops Menu

Lets you select unit operations for the development of a flowsheet. It consists of many hierarchical submenus that group similar unit operations together.
Windows Menu

Executive Report
Displays key information about the material balances and the economic evaluation of the currently active design case. Not available in the current version of BioDesigner.

Stream Report
Displays the stream report of the currently active design case.
Economic Results
Displays the economic analysis report of the currently active design case.

Help Menu

How to use BioDesigner...
Displays information on how to use BioDesigner.

Unit Operations...
Displays information about the various unit operations that are available in the current version of BioDesigner.

Cell Composition
Displays and lets you edit cell composition information about various microorganisms.

Media Preparation
Displays and lets you edit media preparation information.

RawMat Prices
Displays and lets you edit raw material prices information.
APPENDIX II

Objects and Production Rules in the Knowledge Bases of BioDesigner
APPENDIX II: Rules and Objects in BioDesigner

The following pages are print outs of two knowledge bases, one that carries out synthesis of upstream sections and another one that carries out synthesis of downstream sections. This is the format that Nexpert Object stores a knowledge base.

II.1 Knowledge Base that carries out Synthesis of Upstream Section

(@VERSION= 011)
(@PROPERTY= Bead_Shear_Tolerance @TYPE=String;)
(@PROPERTY= Contamination @TYPE=String;)
(@PROPERTY= Enzyme_Cost @TYPE=String;)
(@PROPERTY= Enzyme_Inhibition @TYPE=String;)
(@PROPERTY= Enzyme_In_Final_Product @TYPE=String;)
(@PROPERTY= Formation_Patter @TYPE=String;)
(@PROPERTY= Formation_Pattern @TYPE=String;)
(@PROPERTY= Immobilized_Cells @TYPE=Boolean;)
(@PROPERTY= Location @TYPE=String;)
(@PROPERTY= M W @TYPE=Float;)
(@PROPERTY= Operation @TYPE=String;)
(@PROPERTY= OperMode @TYPE=String;)
(@PROPERTY= Reaction_Is_Aerobic @TYPE=Boolean;)
(@PROPERTY= theName @TYPE=String;)
(@PROPERTY= Type @TYPE=String;)
(@PROPERTY= UnitID @TYPE=Integer;)
(@PROPERTY= UnitName @TYPE=String;)
(@PROPERTY= UnitPurpose @TYPE=String;)
Immoblized_Cells
Operation
OperMode
Reaction_Is_Aerobic
Type
UnitID
UnitName
UnitPurpose

)

(CLASS= Compressor
(PROPERTIES=
OperMode
UnitID
UnitName
UnitPurpose

)

(CLASS= EnzReactor
(SUBCLASSES= Reactor

(PROPERTIES=
Bead_Shear_Tolerance
Contamination
Enzyme_Cost
Enzyme_Inhibition
Enzyme_In_Final_Product
Immoblized_Cells
Operation
OperMode
Reaction_Is_Aerobic
Type
UnitID
UnitName
UnitPurpose

)

(CLASS= HeatSterilizer
(PROPERTIES=
OperMode
UnitID
UnitName
UnitPurpose

)

(CLASS= MembraneSterilizer
(PROPERTIES=
OperMode
UnitID

-215-
UnitName
UnitPurpose

)

(@CLASS= Mixer
 (@PROPERTIES=
    OperMode
    UnitID
    UnitName
    UnitPurpose
  )
)

(@CLASS= Reactor
 (@SUBCLASSES=
    CellReactor
    EnzReactor
  )
 (@PROPERTIES=
    Bead_Shear_Tolerance
    Contamination
    Enzyme_Cost
    Enzyme_Inhibition
    Enzyme_In_Final_Product
    Immobilized_Cells
    Operation
    OperMode
    Reaction_Is_Aerobic
    Type
    UnitID
    UnitName
    UnitPurpose
  )
)

(@CLASS= Sterilizer
 (@SUBCLASSES=
    HeatSterilizer
    MembraneSterilizer
  )
 (@PROPERTIES=
    OperMode
    UnitID
    UnitName
    UnitPurpose
  )
)

(@CLASS= Tank
 (@PROPERTIES=
    OperMode
    UnitID
    UnitName

-216-
UnitPurpose

)

(CLASS= UnitOp
   (SUBCLASSES= Reactor
                Tank
                Sterilizer
                Compressor
                AirFilter
                Mixer
   )
   (Properties=
                OperMode
                UnitID
                UnitName
                UnitPurpose
   )
)

(OBJECT= AirFilterID
   (Properties=
                Value @TYPE=Integer;
   )
)

(OBJECT= Air_Compressor
   (Properties=
                UnitID
                UnitPurpose
   )
)

(OBJECT= AIR_COMPRESSOR_SELECTED
   (Properties=
                Value @TYPE=Boolean;
   )
)

(OBJECT= Air_Inlet_Filter
   (Properties=
                UnitID
                UnitPurpose
   )
)

(OBJECT= Air_Inlet_Filter_Desired
   (Properties=
                Value @TYPE=Boolean;
   )
)

(OBJECT= AIR_INLET_FILTER_SELECTED
(OBJECT= Cell
  (PROPERTIES=
    theName
    Type
  )
)

(OBJECT= Cell_Reactor
  (PROPERTIES=
    Bead_Shear_Tolerance
    Contamination
    Immobilized_Cells
    Operation
    OperMode
    Reaction_Is_Aerobic
    UnitID
    UnitName
    UnitPurpose
  )
)

(OBJECT= CompressorID
  (PROPERTIES=
    Value @TYPE=Integer;
  )
)

(OBJECT= COMPRESSOR_SELECTED
  (PROPERTIES=
    Value @TYPE=Boolean;
  )
)

(OBJECT= Cont_Heat_Sterilizer
  (PROPERTIES=
    UnitID
    UnitPurpose
  )
)

(OBJECT= CONT_HEAT_STERILIZER_SELECTED
  (PROPERTIES=
    Value @TYPE=Boolean;
  )
)

(OBJECT= CONT.IMMOB.GROWING_CELL.REACTOR
  (PROPERTIES=
    Value @TYPE=Boolean;
  )
)

(OBJECT= CONT.IMMOB_NON.GROWING_CELL.REACTOR
  (PROPERTIES= 

  -219-
Value @TYPE=Boolean;
)
)

@OBJECT= CONT_SOLUBLE_ENZYME_REACTOR
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

@OBJECT= CONT_SUSP_GROWING_CELL_REACTOR
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

@OBJECT= CONT_WELL_MIXED_IMMOB_ENZ_REACTOR
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

@OBJECT= Energy_Coupling
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

@OBJECT= Enzyme_Reactor
  (@PROPERTIES=
    Bead_Shear_Tolerance
    Enzyme_Cost
    Enzyme_Inhibition
    Enzyme_In_Final_Product
    Operation
    UnitPurpose
  )
)

@OBJECT= FB_ReactorID
  (@PROPERTIES=
    Value @TYPE=Integer;
  )
)

@OBJECT= FermentorID
  (@PROPERTIES=
    Value @TYPE=Integer;
  )
)

@OBJECT= FILTER_STERILIZER_SELECTED
  (@PROPERTIES=
    Value @TYPE=Boolean;
(OBJECT= First_Task
 (PROPERTIES=
   Value @TYPE=String;
 )
 )

(OBJECT= Gaseous_NH3_is_used_as_Nitrogen_Source
 (PROPERTIES=
   Value @TYPE=Boolean;
 )
 )

(OBJECT= GET_UPSTREAM_DATA
 (PROPERTIES=
   Value @TYPE=Boolean;
 )
 )

(OBJECT= IMMOBIL_ENZYME_REACTOR
 (PROPERTIES=
   Value @TYPE=Boolean;
 )
 )

(OBJECT= MF_for_Sterilizer_Is_Desired
 (PROPERTIES=
   Value @TYPE=Boolean;
 )
 )

(OBJECT= MF_Sterilizer
 (PROPERTIES=
   UnitID
   UnitPurpose
 )
 )

(OBJECT= MicrofilterID
 (PROPERTIES=
   Value @TYPE=Integer;
 )
 )

(OBJECT= Mixer_2ID
 (PROPERTIES=
   Value @TYPE=Integer;
 )
 )

(OBJECT= NH3_Mixer
 (PROPERTIES=

-221-
UnitID
UnitPurpose

)

( OBJECT = NH3_MIXER_SELECTED
  (@PROPERTIES =
    Value @TYPE = Boolean;
  )
)

( OBJECT = NON_GROWING_CELL_REACTOR
  (@PROPERTIES =
    Value @TYPE = Boolean;
  )
)

( OBJECT = PF_ReactorID
  (@PROPERTIES =
    Value @TYPE = Integer;
  )
)

( OBJECT = PLUG_FLOW_IMMOB_ENZ_REACTOR
  (@PROPERTIES =
    Value @TYPE = Boolean;
  )
)

( OBJECT = PLUG_FLOW_REACTOR_SELECTED
  (@PROPERTIES =
    Value @TYPE = Boolean;
  )
)

( OBJECT = Product
  (@PROPERTIES =
    Formation_Pattern
    Location
    MW
    theName
    Type
  )
)

( OBJECT = Reaction_Steps_Less_Than_3
  (@PROPERTIES =
    Value @TYPE = Boolean;
  )
)

( OBJECT = SELECT_AL_FERMENTOR
  (@PROPERTIES =
    Value @TYPE = Boolean;
  )
(OBJECT= STIRRED_FERMENTOR_SELECTED
   (PROPERTIES=
     Value @TYPE=Boolean;
   )
)

(OBJECT= SUSP_GROWING_CELL_REACTOR
   (PROPERTIES=
     Value @TYPE=Boolean;
   )
)

(OBJECT= theREACTOR
   (CLASSES=
     Reactor
   )
   (PROPERTIES=
     Bead_Shear_Tolerance
     Contamination
     Enzyme_Cost
     Enzyme_Inhibition
     Enzyme_In_Final_Product
     Immobilized_Cells
     Operation
     OperMode
     Reaction_Is_Aerobic
     Type
     UnitID
     UnitName
     UnitPurpose
   )
)

(OBJECT= Use_of_Air_Lift_Fermentor_Preferred
   (PROPERTIES=
     Value @TYPE=Boolean;
   )
)

(OBJECT= Use_of_Continuous_Sterilizer_Desired
   (PROPERTIES=
     Value @TYPE=Boolean;
   )
)

(OBJECT= Use_of_Enzyme_Reactor_Desired
   (PROPERTIES=
     Value @TYPE=Boolean;
   )
)
(OBJECT= Use_of_Non_Growing_Cell_Reactor_Desired
  (PROPERTIES=
    Value @TYPE=Boolean;
  )
)

(OBJECT= WELL_MIXED.REACTORSELECTED
  (PROPERTIES=
    Value @TYPE=Boolean;
  )
)

(OBJECT= WM_ReactorID
  (PROPERTIES=
    Value @TYPE=Integer;
  )
)

(SLOT= Air_Inlet_Filter_Desired
  @PROMPT="Is the use of an Air Inlet Filter desired ?";
)

(SLOT= AIR_INLET_FILTERSELECTED
  (CONTEXTS=
    AIR_OUTLET_FILTERSELECTED
  )
)

(SLOT= Air_Outlet_Filter_Desired
  @PROMPT="Is the use of an Air Outlet Filter desired ?";
)

(SLOT= Cell.theName
  @PROMPT="What is the Name of the Cell ?";
)

(SLOT= Cell.Type
  @PROMPT="What is the Cell Type ?";
)

(SLOT= Cell_Reactor.Contamination
  @PROMPT="When contamination takes place is Acute or not ?";
)

(SLOT= Cell_Reactor.Immobilized_Cells
  @PROMPT="Is the use of Immobilized Cells desired ?";
)

(SLOT= Cell_Reactor.Operation
  @PROMPT="Select a mode of operation for Cell Reactor ?";
)

(SLOT= Cell_Reactor.Reaction_Is_Aerobic
  @PROMPT="Is the fermentation Aerobic ?";
)

-225-
(SLOT= Energy_Coupling
    @PROMPT="Do product formation reactions require energy coupling ?";
)

(SLOT= Enzyme_Reactor.Bead_Shear_Tolerance
    @PROMPT="The shear tolerance of enzyme beads is (High or Low) ?";
)

(SLOT= Enzyme_Reactor.Enzyme_Cost
    @PROMPT="Is the cost of enzyme (High or Low) ?";
)

(SLOT= Enzyme_Reactor.Enzyme_In_Final_Product
    @PROMPT="Is the presence of enzyme allowed in the final product or not ?";
)

(SLOT= Enzyme_Reactor.Operation
    @PROMPT="What is the mode of operation of the enzyme reactor ?";
)

(SLOT= Gaseous_NH3_is_used_as_Nitrogen_Source
    @PROMPT="Would you like to use NH3 as Nitrogen source ?";
)

(SLOT= MF_for_Sterilizer_Is_Desired
    @PROMPT="Would you like to use a Microfilter to sterilize the feed to enzyme reactor ?";
)

(SLOT= Product.Formation_Pattern
    @PROMPT="What is the product formation pattern ?";
)

(SLOT= Product.Location
    @PROMPT="What is the location of the product ?";
)

(SLOT= Product.MW
    @PROMPT="What is the molecular weight of the product ?";
)

(SLOT= Product.theName
    @PROMPT="What is the name of the product ?";
)

(SLOT= Product.Type
    @PROMPT="What is the type of the product ?";
)
(SLOT Reaction_Steps_Less_Than_3
   PROMPT="Is the number of reactions steps required for product formation less than 3 ?");

(SLOT START_UPSTREAM_SYNTHESIS
   PROMPT="Would you like to start synthesis of Upstream section ?");

(SLOT Use_of_Air_Lift_Fermentor_Preferred
   PROMPT="Is the use of an Air Lift Fermentor preferred instead of a stirred one ?");

(SLOT Use_of_Continuous_Sterilizer_Desired
   PROMPT="Is the use of a continuous sterilizer desired ?");

(SLOT Use_of_Enzyme_Reactor_Desired
   PROMPT="Is the use of an enzyme reactor for product formation preferred over a cell reactor ?");

(SLOT Use_of_Non_Growing_Cell_Reactor_Desired
   PROMPT="Is the use of a Not Growing Cell Reactor desired ?");

(RULE R1
   INFCAT=45;
   LHS=(Yes (Select_Auxiliary))
       (Is (theREACTOR.Type) (Cell_Reactor))
       (Yes (Cell_Reactor.Reaction_Is_Aerobic))
   )
   HYPO=AIR_COMPRESSOR_SELECTED
   RHS=(CreateObject (Air_Compressor) (1Compressor))
       (Do (CompressorID) (Air_Compressor.UnitID))
       (Let (Air_Compressor.UnitPurpose) (Air_Supply))
       (Show (:BioApropos:Compressor_Selected")
       (KEEP=TRUE;WAIT=TRUE;))
)

(RULE R2
   INFCAT=45;
   LHS=(Yes (AIR_COMPRESSOR_SELECTED))
       (Yes (Air_Inlet_Filter_Desired))
   )
   HYPO=AIR_INLET_FILTER_SELECTED
   RHS=(CreateObject (Air_Inlet_Filter) (1AirFilter))
       (Do (AirFilterID) (Air_Inlet_Filter.UnitID))

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(Let (Air_Inlet_Filter.UnitPurpose) ("In_AirFilter"))
(Show ("BioApropos:AirFilter_In_Selected")
(@KEEP=TRUE;@WAIT=TRUE;))
)

(RULE= R3
@INFCAT=40;
(LHS=
(Is (theREACTOR.Type) ("Cell_Reactor"))
(Yes (Cell_Reactor.Reaction_Is_Aerobic))
(Yes (Air_Outlet_Filter_Desired))
)
(HYPO= AIR_OUTLET_FILTERSELECTED)
(RHS=
(CreateObject (Air_Outlet_Filter) (l_AirFilter1))
(Do (AirFilterID) (Air_Outlet_Filter.UnitID))
(Let (Air_Outlet_Filter.UnitPurpose) ("Out_AirFilter")
(Show ("BioApropos:AirFilter_Out_Selected")
(@KEEP=TRUE;@WAIT=TRUE;))
)
)

(RULE= R4
(LHS=
(Yes (SELECT_ALFERMENTOR))
)
(HYPO= ALFERMENTORSELECTED)
(RHS=
(Do (AL_FermentorID) (theREACTOR.UnitId))
(Let (theREACTOR.UnitPurpose) ("Fermentation")
(Let (Select_Auxiliary) (TRUE))
(Show ("BioApropos:AL_Fermentor_Selected")
(@KEEP=TRUE;@WAIT=TRUE;))
)
)

(RULE= R5
(LHS=
(Yes (SOLUBLE_ENZYME.REACTOR))
(Is (Enzyme_Reactor.Operation) ("Batch"))
)
)
(HYPO= BATCH_SOLUBLE_ENZYME.REACTOR)
)

(RULE= R7
(LHS=
(Yes (SUSP_GROWING_CELL.REACTOR))
(Is (Cell_Reactor.Contamination) ("Acute"))
)
)
(HYPO= BATCH_SUSP_GROWING_CELL.REACTOR)
)

(RULE= R6
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(\textbf{LHS}=
\begin{itemize}
\item \textbf{Yes} (\texttt{SUSP\_GROWING\_CELL\_REACTOR})
\item \textbf{Is} (\texttt{Cell\_Reactor.Contamination}) ("Non\_Acute")
\item \textbf{Is} (\texttt{Cell\_Reactor.Operation}) ("Batch")
\end{itemize}
\textbf{HYPO} = \texttt{BATCH\_SUSP\_GROWING\_CELL\_REACTOR})

(\textbf{RULE} = \texttt{R9}
\textbf{LHS}=
\begin{itemize}
\item \textbf{Yes} (\texttt{Select\_Auxiliary})
\item \textbf{Is} (\texttt{theREACTOR.Type}) ("Cell\_Reactor")
\item \textbf{Is} (\texttt{Cell\_Type}) ("Bacterial")
\item \textbf{Yes} (\texttt{Use\_of\_Continuous\_Sterilizer\_Desired})
\end{itemize}
\textbf{HYPO} = \texttt{CONT\_HEAT\_STERILIZER\_SELECTED})
\textbf{RHS}=
\begin{itemize}
\item (\texttt{CreateObject (Cont\_Heat\_Sterilizer) (1\_Heat\_Sterilizer)})
\item (\texttt{Do (SterilizerID) (Cont\_Heat\_Sterilizer.UnitID)})
\item (\texttt{Let (Cont\_Heat\_Sterilizer.UnitPurpose) ("Sterilization")})
\item (\texttt{Show ("BioApropos:HeatSterilizer\_Selected")})
\end{itemize}
\textbf{KEEP}=\texttt{TRUE}; \textbf{WAIT}=\texttt{TRUE};)

(\textbf{RULE} = \texttt{R8}
\textbf{LHS}=
\begin{itemize}
\item \textbf{Yes} (\texttt{Select\_Auxiliary})
\item \textbf{Is} (\texttt{theREACTOR.Type}) ("Cell\_Reactor")
\item \textbf{Is} (\texttt{Cell\_Type}) ("Yeast")
\item \textbf{Yes} (\texttt{Use\_of\_Continuous\_Sterilizer\_Desired})
\end{itemize}
\textbf{HYPO} = \texttt{CONT\_HEAT\_STERILIZER\_SELECTED})
\textbf{RHS}=
\begin{itemize}
\item (\texttt{CreateObject (Cont\_Heat\_Sterilizer) (1\_Heat\_Sterilizer)})
\item (\texttt{Do (SterilizerID) (Cont\_Heat\_Sterilizer.UnitID)})
\item (\texttt{Let (Cont\_Heat\_Sterilizer.UnitPurpose) ("Sterilization")})
\item (\texttt{Show ("BioApropos:HeatSterilizer\_Selected")})
\end{itemize}
\textbf{KEEP}=\texttt{TRUE}; \textbf{WAIT}=\texttt{TRUE};)

(\textbf{RULE} = \texttt{R10}
\textbf{LHS}=
\begin{itemize}
\item \textbf{Yes} (\texttt{SELECT\_CELL\_REACTOR})
\item \textbf{Is} (\texttt{Product\_Type}) ("A\_Molecule")
\item \textbf{Is} (\texttt{Product\_Location}) ("Extracellular")
\item \textbf{Is} (\texttt{Product\_Formation\_Pattern}) ("Growth\_Associated")
\item \textbf{Yes} (\texttt{Cell\_Reactor\_Immobilized\_Cells})
\item \textbf{Is} (\texttt{Cell\_Reactor\_Operation}) ("Continuous")
\end{itemize}
\textbf{HYPO} = \texttt{CONT\_IMMOB\_GROWING\_CELL\_REACTOR})
(@RULE= R11
  (@LHS=
    (Yes (NON_GROWING_CELL.REACTOR))
    (Yes (Cell_Reactor.Immobilized_Cells))
    (Is (Cell_Reactor.Operation)  ("Continuous"))
  )
  (@HYPO= CONT_IMMOB_NON_GROWING_CELL.REACTOR)
 )

(@RULE= R12
  (@LHS=
    (Yes (SOLUBLE_ENZYME.REACTOR))
    (Is (Enzyme_Reactor.Operation)  ("Continuous"))
  )
  (@HYPO= CONT_SOLUBLE_ENZYME.REACTOR)
 )

(@RULE= R13
  (@LHS=
    (Yes (SUSP_GROWING_CELL.REACTOR))
    (Is (Cell_Reactor.Contamination)  ("Non_Acute"))
    (Is (Cell_Reactor.Operation)  ("Continuous"))
  )
  (@HYPO= CONT_SUSP_GROWING_CELL.REACTOR)
 )

(@RULE= R14
  (@LHS=
    (Yes (IMMOBIL_ENZYME.REACTOR))
    (Is (Enzyme_Reactor.Enzyme_Inhibition)  ("By_Substrate"))
  )
  (@HYPO= CONT_WELL_MIXED_IMMOB_ENZ.REACTOR)
 )

(@RULE= R43
  (@LHS=
    (Yes (Select_Auxiliary))
    (Is (theREACTOR.Type)  ("Enzyme_Reactor"))
    (Yes (MF_for_Sterilizer_Is_Desired))
  )
  (@HYPO= FILTER_STERILIZER_SELECTED)
  (@RHS=
    (CreateObject (MF_Sterilizer)  (1 MembraneSterilizer!))
    (Do (MicrofilterID)  (MF_Sterilizer.UnitID))
    (Let (MF_Sterilizer.UnitPurpose)  ("Sterilization"))
    (Show ("::BioApropos:MF_Sterilizer_Selected")
    )
  )
  (@KEEP=TRUE,@WAIT=TRUE;)
 )

(@RULE= R15
  (@LHS=
    (Yes (Select_Auxiliary))
    (Is (theREACTOR.Type)  ("Cell_Reactor"))
  )

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(Is (Cell.Type) ("Animal"))
)
(@HYPO= FILTER_STERILIZER_SELECTED)
(@RHS=
  (CreateObject (MF_Sterilizer) (1MembraneSterilizer1))
  (Do (MicrofilterID) (MF_Sterilizer.UnitID))
  (Let (MF_Sterilizer.UnitPurpose) ("Sterilization"))
  (Show ("BioApropos:MF_Sterilizer_Selected")
  (@KEEP=TRUE;@WAIT=TRUE;))
)
)

(@RULE= R16
 (@LHS=
  (Yes (START_UPSTREAM_SYNTHESIS))
  (Name (Product.theName) (Product.theName))
 )
 (@HYPO= GET_UPSTREAM_DATA)
 (@RHS=
  (Do (2000) (FermentorID))
  (Do (2014) (AL_FermentorID))
  (Do (2010) (WM_ReactorID))
  (Do (2020) (PF_ReactorID))
  (Do (2030) (FB_ReactorID))
  (Do (2224) (CompressorID))
  (Do (2204) (SterilizerID))
  (Do (2120) (AirFilterID))
  (Do (2210) (Mixer_2ID))
  (Do (2190) (BL_TankID))
  (Do (2090) (MicrofilterID))
  (Let (First_Task) ("Reactor_Selection"))
 )
)

(@RULE= R18
 (@LHS=
  (Yes (SELECT_ENZYME.REACTOR))
  (Is (Enzyme_Reactor.Enzyme_Cost) ("High"))
 )
 (@HYPO= IMMOBIL_ENZYME_REACTOR)
)

(@RULE= R17
 (@LHS=
  (Yes (SELECT_ENZYME.REACTOR))
  (Is (Enzyme_Reactor.Enzyme_In_Final_Product) ("Not_Allowed"))
 )
 (@HYPO= IMMOBIL_ENZYME_REACTOR)
)

(@RULE= R19
 @INFCAST=50;
 (@LHS=
  (Yes (Select_Auxiliary))
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(Is (theREACTOR.Type) ("Cell_Reactor"))
(IsNot (Cell.Type) ("Animal"))
(Yes (Gaseous_NH3_is_used_as_Nitrogen_Source))
)

(@HYPO= NH3_MIXER SeleCTED)
(@RHS=
  (CreateObject (NH3_Mixer) (1Mixer1))
  (Do (Mixer_2ID) (NH3_Mixer.UnitID))
  (Let (NH3_Mixer.UnitPurpose) ("NH3_Supply"))
)

)

(@RULE= R20
(@LHS=
  (Yes (SELECT_CELL_REACTOR))
  (Is (Product.Type) ("A_Molecule"))
  (Is (Product.Location) ("Extracellular"))
  (Is (Product.Formation_Pattern) ("Non_Growth_Associated"))
  (Yes (Use_of_Non_Growing_Cell_Reactor_Desired))
)

 (@HYPO= NON_GROWING_CELL_REACTOR)
)

(@RULE= R21
(@LHS=
  (Yes (IMMOBIL_ENZYME_REACTOR))
  (Is (Enzyme_Reactor.Enzyme_Inhibition) ("By_Product"))
)

 (@HYPO= PLUG_FLOW_IMMOB_ENZ_REACTOR)
)

(@RULE= R22
(@LHS=
  (Yes (SELECT_PF_REACTOR))
)

 (@HYPO= PLUG_FLOW_REACTOR SELECTED)
(@RHS=
  (Do (PF_ReactorID) (theREACTOR.UnitID))
  (Let (theREACTOR.UnitPurpose) ("Product_Formation"))
  (Let (Select_Auxiliary) (TRUE))
  (Show (":BioApropos:PF_Reactor_Selected")
  (@KEEP=TRUE;@WAIT=TRUE;))
)

)

(@RULE= R25
(@LHS=
  (Yes (CONT_WELL_MIXED_IMMOB_ENZ_REACTOR))
  (Is (Enzyme_Reactor.Bead_Shear_Tolerance) ("Low"))
)

 (@HYPO= SELECT_ALFERMENTOR)
)

(@RULE= R24

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(@LHS= 
  (Yes (CONT_IMMOB_GROWING_CELL_REACTOR))
  (Is (Cell_Reactor.Bead_Shear_Tolerance)  
      ("Low")))
)
(@HYPO= SELECT_AL_FERMENTOR)
)

(@RULE= R23
 (@LHS= 
   (Yes (SELECT_CELL_REACTOR))
   (Is (Product.Type)  
       ("Biomass"))
   (Yes (Use_of_Air_Lift_Fermentor_Preferred))
  )
)
(@HYPO= SELECT_AL_FERMENTOR)
)

(@RULE= R26
 (@LHS= 
   (No (SELECT_ENZYME_REACTOR))
  )
)
(@HYPO= SELECT_CELL_REACTOR)
(@RHS= 
  (CreateObject (Cell_Reactor)  
   (CellReactor1))
  (Let (theREACTOR.Type)  
       ("Cell_Reactor"))
  )
)

(@RULE= R27
 (@LHS= 
   (Is (First_Task)  
       ("Reactor_Selection"))
   (Yes (Reaction_Steps_Less_Than_3))
   (No (Energy_Coupling))
   (Is (Product.Type)  
       ("A_Molecule"))
   (Show ("BioApropos:Suggest_EnzReactor")
     (@KEEP=TRUE;@WAIT=TRUE;))
   (Yes (Use_of_Enzyme_Reactor_Desired))
  )
)
(@HYPO= SELECT_ENZYME_REACTOR)
(@RHS= 
  (CreateObject (Enzyme_Reactor)  
   (EnzReactor1))
  (Let (theREACTOR.Type)  
       ("Enzyme_Reactor"))
  )
)

(@RULE= R29
 (@LHS= 
   (Yes (CONT_IMMOB_NON_GROWING_CELL_REACTOR))
  )
)
(@HYPO= SELECT_PF.REACTOR)
)

(@RULE= R28
 (@LHS= 
   (Yes (PLUG_FLOW_IMMOB_ENZ_REACTOR))
  )
)

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(HYPO= SELECT_PF.REACTOR)

(RULE= R31
  (LHS=
    (Yes (BATCH_SUSP_GROWING_CELL.REACTOR))
  )
  (HYPO= SELECT_STIRRED.FERMENTOR)
)

(RULE= R30
  (LHS=
    (Yes (CONT_SUSP_GROWING_CELL.REACTOR))
  )
  (HYPO= SELECT_STIRRED.FERMENTOR)
)

(RULE= R35
  (LHS=
    (Yes (CONT_IMMORB_GROWING_CELL.REACTOR))
    (Is (Cell.REACTor.Bead_Shear_Tolerance) ("High"))
  )
  (HYPO= SELECT_WM.REACTOR)
)

(RULE= R34
  (LHS=
    (Yes (CONT_WELL_MIXED_IMMORB_ENZ.REACTOR))
    (Is (Enzyme.REACTor.Bead_Shear_Tolerance) ("High"))
  )
  (HYPO= SELECT_WM.REACTOR)
)

(RULE= R33
  (LHS=
    (Yes (CONT_SOLUBLE_ENZYME.REACTOR))
  )
  (HYPO= SELECT_WM.REACTOR)
)

(RULE= R32
  (LHS=
    (Yes (BATCH_SOLUBLE_ENZYME.REACTOR))
  )
  (HYPO= SELECT_WM.REACTOR)
)

(RULE= R36
  (LHS=
    (Yes (SELECT_ENZYME.REACTOR))
    (Is (Enzyme.REACTor.Enzyme_Cost) ("Low"))
    (Is (Enzyme.REACTor.Enzyme_In_Final_Product) ("Allowed"))
  )
)
(@HYPO = SOLUBLE_ENZYME.REACTOR)
)

(@RULE = R37
 (@LHS =
    (Yes (SELECT_STIRRED.FERMENTOR))
  )
 (@HYPO = STIRRED.FERMENTOR_SELECTED)
 (@RHS =
    (Do (FermentorID) (theREACTION.UnitID))
    (Let (theREACTION.UnitPurpose) ("Fermentation"))
    (Let (Select.Auxiliary) (TRUE))
    (Show ("BioApropos:Fermentor_Selected")
  )
 (@KEEP = TRUE; @WAIT = TRUE;)
)

(@RULE = R40
 (@LHS =
    (Yes (SELECT_CELL.REACTOR))
    (Is (Product.Type) ("A.Molecule"))
    (Is (Product.Location) ("Extracellular"))
    (Is (Product.Formation.Pattern) ("Growth_Associated"))
  )
 (@HYPO = SUSP.GROWING.CELL.REACTOR)
)

(@RULE = R39
 (@LHS =
    (Yes (SELECT_CELL.REACTOR))
    (Is (Product.Type) ("A.Molecule"))
    (Is (Product.Location) ("Intracellular"))
  )
 (@HYPO = SUSP.GROWING.CELL.REACTOR)
)

(@RULE = R38
 (@LHS =
    (Yes (SELECT_CELL.REACTOR))
    (Is (Product.Type) ("Biomass"))
    (No (Use_of_Air.Lift.Fermentor_Preferred))
  )
 (@HYPO = SUSP.GROWING.CELL.REACTOR)
)

(@RULE = R41
 (@LHS =
    (Yes (SELECT_WM.REACTOR))
  )
 (@HYPO = WELL_MIXED.REACTOR_SELECTED)
 (@RHS =
    (Do (WM_ReactorID) (theREACTION.UnitID))
    (Let (theREACTION.UnitPurpose) ("Product.Formation"))
    (Let (Select.Auxiliary) (TRUE))
  )
(Show (".BioApropos:WM_Reactor_Selected")
(@KEEP=TRUE;@WAIT=TRUE;))
)

(@GLOBALS=
 @INHVALUP=FALSE;
 @INHVALDOWN=TRUE;
 @INHOBJUP=FALSE;
 @INHOBJDOWN=FALSE;
 @INHCCLASSUP=FALSE;
 @INHCCLASSDOWN=TRUE;
 @INHBRADTH=TRUE;
 @INHPARENT=FALSE;
 @PWTRUE=TRUE;
 @PWFALSE=TRUE;
 @PWNOTKNOWN=TRUE;
 @EXHBWRD=TRUE;
 @PTGATES=TRUE;
 @PFACIONS=TRUE;
 @SOURCESON=TRUE;
 @CACTIONSON=TRUE;
 @SUGLIST=GET_UPSTREAM_DATA;
)

II.2 Knowledge Base that carries out Synthesis of Downstream Section

(@VERSION= 011)
(@PROPERTY= Activity_State @TYPE=String;)
(@PROPERTY= Density @TYPE=Float;)
(@PROPERTY= Diameter @TYPE=Float;)
(@PROPERTY= Formation @TYPE=String;)
(@PROPERTY= Heat_Resistant @TYPE=Boolean;)
(@PROPERTY= Location @TYPE=String;)
(@PROPERTY= Morphology_Mycelial @TYPE=Boolean;)
(@PROPERTY= MW @TYPE=Float;)
(@PROPERTY= Required_Phase @TYPE=String;)
(@PROPERTY= Required_Purity @TYPE=String;)
(@PROPERTY= theName @TYPE=String;)
(@PROPERTY= Type @TYPE=String;)
(@PROPERTY= UnitID @TYPE=Integer;)
(@PROPERTY= UnitName @TYPE=String;)
(@PROPERTY= UnitUse @TYPE=String;)

(@CLASS= UNIT_OPS
 (@PROPERTIES=
   UnitID
   UnitName

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(OBJECT= Affinity_ChromID
   (PROPERTIES=
      Value @TYPE=Integer;
   )
)

(OBJECT= Altern_for_Buffer_Exchange
   (PROPERTIES=
      Value @TYPE=String;
   )
)

(OBJECT= Altern_for_Cell_Disruption
   (PROPERTIES=
      Value @TYPE=String;
   )
)

(OBJECT= Altern_for_Cell_Harvesting
   (PROPERTIES=
      Value @TYPE=String;
   )
)

(OBJECT= Bead_MillID
   (PROPERTIES=
      Value @TYPE=Boolean;
   )
)

(OBJECT= Biomass_Pathway
   (PROPERTIES=
      Value @TYPE=Boolean;
   )
)

(OBJECT= Biomass_RecoveryDone
   (PROPERTIES=
      Value @TYPE=Boolean;
   )
)

(OBJECT= Biomass_RemovalDone
   (PROPERTIES=
      Value @TYPE=Boolean;
   )
)

(OBJECT= Biomass_Required_in_Dried_Form
(PROP
    Value TYPE=Boolean;
)

(OBJECT= BL_TankID
    (PROP
        Value TYPE=Integer;
    )
)

(OBJECT= BML_for_Cell_Disruption
    (PROP
        Value TYPE=Boolean;
    )
)

(OBJECT= BS_CentrifugeID
    (PROP
        Value TYPE=Integer;
    )
)

(OBJECT= Buffer_Exchange_Done
    (PROP
        Value TYPE=Boolean;
    )
)

(OBJECT= Cell
    (PROP
        Density
        Diameter
        Location
        Morphology_Mycelial
    )
)

(OBJECT= Cell_Disruption_Done
    (PROP
        Value TYPE=Boolean;
    )
)

(OBJECT= Cell_Harvesting_Done
    (PROP
        Value TYPE=Boolean;
    )
)

(OBJECT= Comp_SplitterID
    (PROP
        Value TYPE=Integer;
    )
)
(OBJECT= DCF_for_Biomass_Recovery
   (@PROPERTIES=
     Value @TYPE=Boolean;
   )
)

(OBJECT= DCF_for_Biomass_Removal
   (@PROPERTIES=
     Value @TYPE=Boolean;
   )
)

(OBJECT= DCF_for_Cell_Harvesting
   (@PROPERTIES=
     Value @TYPE=Boolean;
   )
)

(OBJECT= DCF_for_Debris_Removal
   (@PROPERTIES=
     Value @TYPE=Boolean;
   )
)

(OBJECT= DCF_for_Incl_Body_Recovery
   (@PROPERTIES=
     Value @TYPE=Boolean;
   )
)

(OBJECT= DDR_for_Biomass_Drying
   (@PROPERTIES=
     Value @TYPE=Boolean;
   )
)

(OBJECT= Debris_Removal.Done
   (@PROPERTIES=
     Value @TYPE=Boolean;
   )
)

(OBJECT= DEF_for_Polishing
   (@PROPERTIES=
     Value @TYPE=Boolean;
   )
)

(OBJECT= DE_FilterID
   (@PROPERTIES=
     Value @TYPE=Integer;
   )

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(OBJECT= DR_DryerID
   (PROPERTIES=
      Value @TYPE=Integer;
   )
)

(OBJECT= DS_CentrifugeID
   (PROPERTIES=
      Value @TYPE=Integer;
   )
)

(OBJECT= Extracellular_Pathway
   (PROPERTIES=
      Value @TYPE=Boolean;
   )
)

(OBJECT= FBD_for_Biomass_Drying
   (PROPERTIES=
      Value @TYPE=Boolean;
   )
)

(OBJECT= FB_Drier_for_Biomass_Drying
   (PROPERTIES=
      Value @TYPE=Boolean;
   )
)

(OBJECT= FB_DryerID
   (PROPERTIES=
      Value @TYPE=Float;
   )
)

(OBJECT= FB_Dryer_for_Solvent_Removal
   (PROPERTIES=
      Value @TYPE=Boolean;
   )
)

(OBJECT= FDR_for_Solvent_Removal
   (PROPERTIES=
      Value @TYPE=Boolean;
   )
)

(OBJECT= Feed_to_Cell_Disruption_less_than_1_m3_per_h
   (PROPERTIES=
      Value @TYPE=Boolean;
   )
)
First_Task
    (@PROPERTIES=
        Value @TYPE=String;
    )

Flocculation_Desired
    (@PROPERTIES=
        Value @TYPE=Boolean;
    )

Flow_SplitterID
    (@PROPERTIES=
        Value @TYPE=Integer;
    )

FR_DryerID
    (@PROPERTIES=
        Value @TYPE=Integer;
    )

Gel_FilterID
    (@PROPERTIES=
        Value @TYPE=Integer;
    )

Gel_Filter_for_Buffer_Exchange
    (@PROPERTIES=
        Value @TYPE=Boolean;
    )

GET_DOWNSTREAM_DATA
    (@PROPERTIES=
        Value @TYPE=Boolean;
    )

Get_More_Data
    (@PROPERTIES=
        Value @TYPE=Boolean;
    )

Get_More_Data_1
    (@PROPERTIES=
        Value @TYPE=Boolean;
    )
@OBJECT= Get_More_Data_2
(@PROPERTIES=
    Value @TYPE[Boolean;
)
)

@OBJECT= Get_More_Data_3
(@PROPERTIES=
    Value @TYPE[Boolean;
)
)

@OBJECT= Get_More_Data_4
(@PROPERTIES=
    Value @TYPE[Boolean;
)
)

@OBJECT= GFL_for_Purification
(@PROPERTIES=
    Value @TYPE[Boolean;
)
)

@OBJECT= Heat_ExchangerID
(@PROPERTIES=
    Value @TYPE[Integer;
)
)

@OBJECT= HMG_for_Cell_Disruption
(@PROPERTIES=
    Value @TYPE[Boolean;
)
)

@OBJECT= HomogenizerID
(@PROPERTIES=
    Value @TYPE[Integer;
)
)

@OBJECT= Incl_Body_Recovery_Done
(@PROPERTIES=
    Value @TYPE[Boolean;
)
)

@OBJECT= Intracellular_Pathway
(@PROPERTIES=
    Value @TYPE[Boolean;
)
(OBJECT= INX_for_Purification
  (PROPERTYs=
    Value @TYPE=Boolean;
  )
)

(OBJECT= Ion_ExchangerID
  (PROPERTYs=
    Value @TYPE=Integer;
  )
)

(OBJECT= MF_for_Cell_Harvesting
  (PROPERTYs=
    Value @TYPE=Boolean;
  )
)

(OBJECT= MF_for_Polishing
  (PROPERTYs=
    Value @TYPE=Boolean;
  )
)

(OBJECT= MicrofilterID
  (PROPERTYs=
    Value @TYPE=Integer;
  )
)

(OBJECT= Mixer_2ID
  (PROPERTYs=
    Value @TYPE=Integer;
  )
)

(OBJECT= Mixer_3ID
  (PROPERTYs=
    Value @TYPE=Integer;
  )
)

(OBJECT= Move_to_Biomass_Recovery
  (PROPERTYs=
    Value @TYPE=Boolean;
  )
)

(OBJECT= Move_to_Cell_Disruption
  (PROPERTYs=
    Value @TYPE=Boolean;
  )
)
Value @TYPE=Boolean;
)
)

(@OBJECT= Product_Forms_Incusion_Bodies
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

(@OBJECT= PumpID
  (@PROPERTIES=
    Value @TYPE=Integer;
  )
)

(@OBJECT= REACTOR
  (@PROPERTIES=
    Type
  )
)

(@OBJECT= RO_for_Product_Concentration
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

(@OBJECT= RVF_for_Biomass_Recovery
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

(@OBJECT= RVF_for_Biomass_Removal
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

(@OBJECT= RV_FilterID
  (@PROPERTIES=
    Value @TYPE=Integer;
  )
)

(@OBJECT= SDR_for_Solvent_Removal
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

(@OBJECT= Selection_Done
  (@PROPERTIES=

  )

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Value @TYPE=Boolean;
)
)

@OBJECT= SP_DryerID
  (@PROPERTIES=
    Value @TYPE=Integer;
  )
)

@OBJECT= Start_Synthesis_of_Downstream_Section
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

@OBJECT= UF_for_Buffer_Exchange
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

@OBJECT= UF_for_Concentration
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

@OBJECT= UF_for_Product_Concentration
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

@OBJECT= UltrafilterID
  (@PROPERTIES=
    Value @TYPE=Integer;
  )
)

@OBJECT= User_Def_SepID
  (@PROPERTIES=
    Value @TYPE=Integer;
  )
)

@OBJECT= Use_of_Bead_Mill_preferred_over_Homogenizer
  (@PROPERTIES=
    Value @TYPE=Boolean;
  )
)

@OBJECT= WMR_for_Flocculation
  (@PROPERTIES=
Value @TYPE=Boolean;
)
)

@OBJECT= WMR_for_Refolding
 (@PROPERTIES=
   Value @TYPE=Boolean;
)
)

@OBJECT= WMR_for_Solubilization
 (@PROPERTIES=
   Value @TYPE=Boolean;
)
)

@OBJECT= WM_ReactorID
 (@PROPERTIES=
   Value @TYPE=Integer;
)
)

@SLOT= Altern_for_Buffer_Exchange
   @PROMPT="What would you like to use for buffer exchange ?";
)

@SLOT= Altern_for_Cell_Disruption
   @PROMPT="What would you like to use for cell disruption ?";
)

@SLOT= Altern_for_Cell_Harvesting
   @PROMPT="What would you like to use for Cell Harvesting ?";
)

@SLOT= Biomass_Required_in_Dried_Form
   @PROMPT="Is biomass required in dried final form ?";
)

@SLOT= Cell.Density
   @PROMPT="What is the Cell Density (in Kg/m3)";
)

@SLOT= Cell.Diameter
   @PROMPT="What is the Cell Diameter (in microns) ";
)

@SLOT= Feed_to_Cell_Disruption_less_than_1_m3_per_h
   @PROMPT="Is Feed to Cell Disruption < 1 m3/h";
)

@SLOT= Flocculation_Desired
   @PROMPT="Is flocculation desired to facilitate biomass recovery ?";
)
(SLOT= Product.Activity.State
  @PROMPT="Is the product produced in Active or Denatured form ?";
)

(SLOT= Product.Heat_Resistant
  @PROMPT="Is the product Heat Resistant ?";
)

(SLOT= Product.Required_Phase
  @PROMPT="What is the required product phase (solid or liquid) ?";
)

(SLOT= Product.Required_Purity
  @PROMPT="Is the final product required in High or Low purity ?";
)

(SLOT= Product.theName
  @PROMPT="What is the name of the product ?";
)

(SLOT= Product_Forms_Inclusion_Bodies
  @PROMPT="Does the product form inclusion bodies ?";
)

(SLOT= REACTOR.Type
  @PROMPT="Is Cell or Enzyme reactor used ?";
)

(SLOT= Start_Synthesis_of_Downstream_Section
  @PROMPT="Start Synthesis of Downstream Section ?";
)

(SLOT= Use_of_Bead_Mill_preferred_over_Homogenizer
  @PROMPT="Is the use of Bead Mill preferred over Homogenizer for cell disruption ?";
)

(RULE= R2
  (@LHS=
    (Yes (DCF_for_Biomass_Recovery))
  )
  (@HYPO= Biomass_Recovery_Done)
)

(RULE= R1
  (@LHS=
    (Yes (RVF_for_Biomass_Recovery))
  )
  (@HYPO= Biomass_Recovery_Done)
)

(RULE= R4
  (@LHS=
    (Yes (RVF_for_Biomass_Removal))
  )
)

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(RH@ = BML_for_Cell_Disruption)

(RH@ = CreateObject ('UnitOp\n\n') (UNIT_OPS))

(LH@ = Let ('UnitOp\n.UnitName) ("Bead_Mill")

(LH@ = Let ('UnitOp\n.UnitUse) ("Cell_Disruption")

(RH@ = Do (Bead_MillID 'UnitOp\n.UnitID))

(RH@ = Do (n+1) (n))

(RH@ = R7)

(RH@ = UF_for_Buffer_Exchange)

(RH@ = Buffer_Exchange.Done)

(RH@ = R6)

(RH@ = Gel_Filter_for_Buffer_Exchange)

(RH@ = Buffer_Exchange.Done)

(RH@ = R9)

(RH@ = BML_for_Cell_Disruption)

(RH@ = Cell_Disruption.Done)

(RH@ = R8)

(RH@ = HMG_for_Cell_Disruption)

(RH@ = Cell_Disruption.Done)
(RULE R11
 (LHS=
 (Yes (MF_for_Cell_Harvesting))
 )
 (HYPO= Cell_HarvestingDone)
 )

(RULE R10
 (LHS=
 (Yes (DCF_for_Cell_Harvesting))
 )
 (HYPO= Cell_HarvestingDone)
 )

(RULE R12
 (LHS=
 (Yes (Move_to_Biomass_Recovery))
 (> (Cell.Diameter) 1.0)
 (> (Cell.Density) 1050)
 )
 (HYPO= DCF_for_Biomass_Recovery)
 (RHS=
 (CreateObject ('UnitOp\n \\n) 'UNIT_OPS!)
 (Let ('UnitOp\n \n.UnitName) "DS_Centrifuge")
 (Let ('UnitOp\n \n.UnitUse) "Biomass_Recovery")
 (Do (DS_CentrifugeID ('UnitOp\n .UnitID))
 (Do (n+1) (n))
 )
 )

(RULE R13
 (LHS=
 (Is (First_Task) "BiomassRemoval")
 (No (Cell.Morphology_Mycelial))
 )
 (HYPO= DCF_for_BiomassRemoval)
 (RHS=
 (CreateObject ('UnitOp\n \n) 'UNIT_OPS!)
 (Let ('UnitOp\n \n.UnitName) "DS_Centrifuge")
 (Let ('UnitOp\n \n.UnitUse) "BiomassRemoval")
 (Do (DS_CentrifugeID ('UnitOp\n .UnitID))
 (Do (n+1) (n))
 )
 )

(RULE R14
 (LHS=
 (Yes (Move_to_Cell_Harvesting))
 (Is (Altern_for_Cell_Harvesting) "DS_Centrifuge")
 )
 (HYPO= DCF_for_Cell_Harvesting)
 (RHS=
 (CreateObject ('UnitOp\n \n) 'UNIT_OPS!)
 (Let ('UnitOp\n .UnitName) "DS_Centrifuge")
 )
 )
(Let ('UnitOp\n\UnitUse) ("Cell_Harvesting"))
(Do (DS_CentrifugeID) ('UnitOp\n\UnitID))
(Do (n+1) (n))
)

(@RULE= R15
(@LHS=
  (Yes (Cell_Disruption_Done))
  (Is (Product.Activity_State) ("Active"))
)
(@HYPO= DCF_for_Debris_Removal)
(@RHS=
  (CreateObject ('UnitOp\n) (!UNIT_OPS!))
  (Let ('UnitOp\n\UnitName) ("DS_Centrifuge")
  (Let ('UnitOp\n\UnitUse) ("Debris_Removal")
  (Do (DS_CentrifugeID) ('UnitOp\n\UnitID))
  (Do (n+1) (n))
)
)

(@RULE= R16
(@LHS=
  (Yes (Cell_Disruption_Done))
  (Is (Product.Activity_State) ("Denatured"))
  (Yes (Product_Forms_Inclusion_Bodies))
)
(@HYPO= DCF_for_Incl_Body_Recovery)
(@RHS=
  (CreateObject ('UnitOp\n) (!UNIT_OPS!))
  (Let ('UnitOp\n\UnitName) ("DS_Centrifuge")
  (Let ('UnitOp\n\UnitUse) ("Incl_BODY_Recoy")
  (Do (DS_CentrifugeID) ('UnitOp\n\UnitID))
  (Do (n+1) (n))
)
)

(@RULE= R17
(@LHS=
  (Yes (DCF_for_Debris_Removal))
)
(@HYPO= Debris_Removal_Done)
)

(@RULE= R18
(@LHS=
  (Yes (Product_Concentration_Done))
  (Is (Product.Location) ("Intracellular"))
  (Is (Product.Activity_State) ("Denatured"))
  (Yes (Product_Forms_Inclusion_Bodies))
  (Is (Product.Required_Purity) ("High"))
)
(@HYPO= DEF_for_Polishing)
(@RHS=}

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(CreateObject ('UnitOp\n' \n) \n "UNIT_OPS\n")
(Let ('UnitOp\n\n.UnitName) \n "DE_Filter")
(Let ('UnitOp\n\n.UnitUse) \n "Polishing")
(Do (DE_FilterID) \n ('UnitOp\n\n.UnitID))
(Do \n (n+1) \n (n))
)

(@RULE= \n R19
(@LHS= \n (Yes \n \n (Biomass_Recovery.Done))
(Yes \n \n (Biomass_Required_in_Dried_Form))
)
(@HYPO= \n FB_Drier_for_Biomass_Drying)
(@RHS= \n (CreateObject ('UnitOp\n' \n) \n "UNIT_OPS\n")
(Let ('UnitOp\n\n.UnitName) \n "FB_Dryer")
(Let ('UnitOp\n\n.UnitUse) \n "Biomass_Drying")
(Do (FB_DryerID) \n ('UnitOp\n\n.UnitID))
(Do \n (n+1) \n (n))
)

(@RULE= \n R20
(@LHS= \n (Yes \n \n (Product_Concentration.Done))
(Is \n \n (Product.Required_Purity) \n \n "Low")
(Is \n \n (Product.Required_Phase) \n \n "Solid")
)
(@HYPO= \n FB_Dryer_for_Solvent_Removal)
(@RHS= \n (CreateObject ('UnitOp\n' \n) \n "UNIT_OPS\n")
(Let ('UnitOp\n\n.UnitName) \n "FB_Dryer")
(Let ('UnitOp\n\n.UnitUse) \n \n "Solvent_Removal")
(Do (FB_DryerID) \n ('UnitOp\n\n.UnitID))
(Do \n (n+1) \n (n))
)

(@RULE= \n R21
(@LHS= "WMR_for_Solubilization)
(Is \n \n (Altern_for_Buffer_Exchange) \n \n "Gel_Filter")
)
(@HYPO= \n Gel_Filter_for_Buffer_Exchange)
(@RHS= \n (CreateObject ('UnitOp\n' \n) \n "UNIT_OPS\n")
(Let ('UnitOp\n\n.UnitName) \n "Gel_Filter")
(Let ('UnitOp\n\n.UnitUse) \n "Buffer_Exchange")
(Do (Gel_FilterID) \n ('UnitOp\n\n.UnitID))
(Do \n (n+1) \n (n))
)

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(RULE=R22
  (LHS=
    (Yes (Start_Synthesis_of_Downstream_Section))
    (Name (Product.theName) (Product.theName))
    (Name (REACTOR.Type) (REACTOR.Type))
  )
  (HYPO= GET_DOWNSTREAM_DATA)
  (RHS=
    (Do (2050) (DS_CentrifugeID))
    (Do (2060) (BS_CentrifugeID))
    (Do (2070) (HomogenizerID))
    (Do (2080) (Bead_MillID))
    (Do (2090) (MicrofilterID))
    (Do (2100) (UltrafilterID))
    (Do (2110) (RV_FilterID))
    (Do (2114) (DE_FilterID))
    (Do (2130) (Gel_FilterID))
    (Do (2140) (Ion_ExchangeID))
    (Do (2160) (Affinity_ChromID))
    (Do (2180) (WM_ReactorID))
    (Do (2190) (BL_TankID))
    (Do (2200) (Heat_ExchangerID))
    (Do (2220) (PumpID))
    (Do (2210) (Mixer_2ID))
    (Do (2215) (Mixer_3ID))
    (Do (2230) (FLO_SplitterID))
    (Do (2240) (Comp_SplitterID))
    (Do (2250) (DR_DryerID))
    (Do (2260) (SP_DryerID))
    (Do (2270) (FR_DryerID))
    (Do (2280) (FB_DryerID))
    (Do (2350) (User_Def_SepID))
    (Do (1) (n))
  )
)

(RULE=R23
  (LHS=
    (Yes (GET_DOWNSTREAM_DATA))
    (Is (REACTOR.Type) ("Cell_Reactor"))
    (Is (Product.Type) ("Biomass"))
  )
  (HYPO= Get_More_Data_1)
  (RHS=
    (Let (First_Task) ("Biomass_Recovery"))
  )
)

(RULE=R24
  (LHS=
    (Yes (GET_DOWNSTREAM_DATA))
    (Is (REACTOR.Type) ("Cell_Reactor"))
    (Is (Product.Type) ("A_Molecule"))
    (Is (Product.Location) ("Intracellular"))
  )
)

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(@RULE= R25
 (@LHS=
 (Yes (GET_DOWNSTREAM_DATA))
 (Is (REACTOR.Type) ("Cell_Reactor")))
 (Is (Product.Type) ("A_Molecule")))
 (@HYPO= Get_More_Data_3)
 (@RHS=
 (Let (First_Task) ("Biomass Removal")))
 )

(@RULE= R26
 (@LHS=
 (Yes (GET_DOWNSTREAM_DATA))
 (Is (REACTOR.Type) ("Enzyme_Reactor")))
 )
 (@HYPO= Get_More_Data_4)
 (@RHS=
 (Let (First_Task) ("Concentration")))
 )

(@RULE= R27
 (@LHS=
 (Yes (INX_for_Purification))
 )
 (@HYPO= GFL_for_Purification)
 (@RHS=
 (CreateObject ('UnitOp\n\n) (UNIT_OPS))
 (Let ('UnitOp\n\n.UnitName) ("Gel_Filler"))
 (Let ('UnitOp\n\n.UnitUse) ("Purification"))
 (Do (Gel_FilterID) ('UnitOp\n\n.UnitID))
 (Do (n+1) (n))
 )
)

(@RULE= R28
 (@LHS=
 (Yes (Move_to_Cell_Disruption))
 (Is (Altern_for_Cell_Disruption) ("Homogenizer")))
 )
 (@HYPO= HMG_for_Cell_Disruption)
 (@RHS=
 (CreateObject ('UnitOp\n\n) (UNIT_OPS))
 (Let ('UnitOp\n\n.UnitName) ("Homogenizer"))

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(Let ('UnitOp\n.UnitUse) ("Cell_Disruption"))
(Do (HomogenizerID) ('UnitOp\n.UnitID))
(Do (n+1) (n))
)

(@RULE= R29
 (@LHS= 
   (Yes (DCF_for_Incl_Body_Recovery))
   )
 (@HYPO= Incl_Body_Recovery.Done)
)

(@RULE= R31
 (@LHS= 
   (Yes (DEF_for_Polishing))
   )
 (@HYPO= INX_for_Purification)
 (@RHS= 
   (CreateObject ('UnitOp\n) ("UNIT_OPS1")
   (Let ('UnitOp\n.UnitName) ("Ion_Exchanger"))
   (Let ('UnitOp\n.UnitUse) ("Purification"))
   (Do (Ion_ExchangerID) ('UnitOp\n.UnitID))
   (Do (n+1) (n))
   )
)

(@RULE= R30
 (@LHS= 
   (Yes (Product_Concentration.Done))
   (Is (Product.Required_Purity) ("High"))
   (No (DEF_for_Polishing))
   )
 (@HYPO= INX_for_Purification)
 (@RHS= 
   (CreateObject ('UnitOp\n) ("UNIT_OPS1")
   (Let ('UnitOp\n.UnitName) ("Ion_Exchanger"))
   (Let ('UnitOp\n.UnitUse) ("Purification"))
   (Do (Ion_ExchangerID) ('UnitOp\n.UnitID))
   (Do (n+1) (n))
   )
)

(@RULE= R32
 (@LHS= 
   (Yes (Move_to_Cell_Harvesting))
   (Is (Altern_for_Cell_Harvesting) ("Microfilter"))
   )
 (@HYPO= MF_for_Cell_Harvesting)
 (@RHS= 
   (CreateObject ('UnitOp\n) ("UNIT_OPS1")
   (Let ('UnitOp\n.UnitName) ("Microfilter"))
   (Let ('UnitOp\n.UnitUse) ("Cell_Harvesting"))
   (Do (MicrofilterID) ('UnitOp\n.UnitID))
   )
)
(Do (n+1) (n))
)

(@RULE= R33
 (@LHS=
   (Yes (Debris_Removal_Done))
   (Is (Product.Required_Purity) ("High"))
 )
 (@HYPO= MF_for_Polishing)
 (@RHS=
   (CreateObject ('UnitOp'\n) (!UNIT_OPS!!))
   (Let ('UnitOp'\n[UnitName] ("Microfilter"))
   (Let ('UnitOp'\n[UnitUse] ("Polishing"))
   (Do (MicrofilterID) ('UnitOp'\n[UnitID])
   (Do (n+1) (n))
 )
)

(@RULE= R34
 (@LHS=
   (No (WMR_for_Flocculation))
   (Is (Product.Type) ("Biomass"))
 )
 (@HYPO= Move_to_Biomass_Recovery)
)

(@RULE= R35
 (@LHS=
   (Yes (WMR_for_Flocculation))
 )
 (@HYPO= Move_to_Biomass_Recovery)
)

(@RULE= R37
 (@LHS=
   (Yes (Cell_Harvesting_DONE))
   (Yes (Feed_to_Cell_Disruption_less_than_1_m3_per_h))
 )
 (@HYPO= Move_to_Cell_Disruption)
)

(@RULE= R36
 (@LHS=
   (Yes (Cell_Harvesting_DONE))
   (No (Feed_to_Cell_Disruption_less_than_1_m3_per_h))
 )
 (@HYPO= Move_to_Cell_Disruption)
 (@RHS=
   (Let (Altern_for_Cell_Disruption) ("Homogenizer"))
 )
)

(@RULE= R55

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(@LHS=
  (Is (First_Task) ("Cell_Harvesting")))
)
(@HYPO= Move_to_Cell_Harvesting)
)

(@RULE= R54
(@LHS=
  (Is (First_Task) ("Cell_Harvesting"))
  (<= (Cell.Diameter) (1.0))
)
(@HYPO= Move_to_Cell_Harvesting)
(@RHS=
  (Let (Altern_for_Cell_Harvesting) ("Microfilter"))
)
)

(@RULE= R53
(@LHS=
  (Is (First_Task) ("Cell_Harvesting"))
  (> (Cell.Diameter) (1.0))
  (> (Cell.Density) (1050))
)
(@HYPO= Move_to_Cell_Harvesting)
(@RHS=
  (Let (Altern_for_Cell_Harvesting) ("DS_Centrifuge"))
)
)

(@RULE= R38
(@LHS=
  (Yes (MF_for_Polishing))
)
(@HYPO= Polishing_of_Intra_Active_Done)
)

(@RULE= R40
(@LHS=
  (Yes (RO_for_Product_Concentration))
)
(@HYPO= Product_Concentration.Done)
)

(@RULE= R39
(@LHS=
  (Yes (UF_for_Product_Concentration))
)
(@HYPO= Product_Concentration.Done)
)

(@RULE= R42
(@LHS=
  (Is (First_Task) ("Concentration"))
  (<= (Product.MW) (10000.0))
)
)

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(\textbf{HYPO} = \text{RO_for_Product_Concentration})
(\textbf{RHS} =
  (\text{CreateObject ('UnitOp'\n}) (\text{\text{UNIT_OPS}1}))
  (\text{Let ('UnitOp'\n[UnitName]) ("Rev_Osmosis"))}
  (\text{Let ('UnitOp'\n[UnitUse]) ("Concentration"))}
  (\text{Do (User_Def_SepID) ('UnitOp'\n[UnitID])}
  (\text{Do (n+1) (n)}))
)

(\textbf{RULE} = \text{R41}
(\textbf{LHS} =
  (\text{Yes (Biomass_Removal_Done))}
  (\text{<= (Product.MW) (10000.0))})
)
(\textbf{HYPO} = \text{RO_for_Product_Concentration})
(\textbf{RHS} =
  (\text{CreateObject ('UnitOp'\n}) (\text{\text{UNIT_OPS}1}))
  (\text{Let ('UnitOp'\n[UnitName]) ("Rev_Osmosis"))}
  (\text{Let ('UnitOp'\n[UnitUse]) ("Concentration"))}
  (\text{Do (User_Def_SepID) ('UnitOp'\n[UnitID])}
  (\text{Do (n+1) (n)}))
)

(\textbf{RULE} = \text{R43}
(\textbf{LHS} =
  (\text{Yes (Move_to_Biomass_Recovery))}
  (\text{No (DCF_for_Biomass_Recovery))})
)
(\textbf{HYPO} = \text{RVF_for_Biomass_Recovery})
(\textbf{RHS} =
  (\text{CreateObject ('UnitOp'\n}) (\text{\text{UNIT_OPS}1}))
  (\text{Let ('UnitOp'\n[UnitName]) ("RV_Filter"))}
  (\text{Let ('UnitOp'\n[UnitUse]) ("Biomass_Recovery"))}
  (\text{Do (RV_FilterID) ('UnitOp'\n[UnitID])}
  (\text{Do (n+1) (n)}))
)

(\textbf{RULE} = \text{R44}
(\textbf{LHS} =
  (\text{Is (First_Task) ("Biomass_Removal"))}
  (\text{Yes (Cell.Morphology_Mycelial))})
)
(\textbf{HYPO} = \text{RVF_for_Biomass_Removal})
(\textbf{RHS} =
  (\text{CreateObject ('UnitOp'\n}) (\text{\text{UNIT_OPS}1}))
  (\text{Let ('UnitOp'\n[UnitName]) ("RV_Filter"))}
  (\text{Let ('UnitOp'\n[UnitUse]) ("Biomass_Removal"))}
  (\text{Do (RV_FilterID) ('UnitOp'\n[UnitID])}
  (\text{Do (n+1) (n)}))
)
(RULE= R45
  (LHS=
    (Yes (WMR_for_Solubilization))
    (Is (Altern_for_Buffer_Exchange) ("Ultrafilter"))
  )
  (HYPO= UF_for_Buffer_Exchange)
  (RHS=
    (CreateObject ('UnitOp\n) (\UNIT_OPS!))
    (Let ('UnitOp\n.UnitName) ("Ultrafilter"))
    (Let ('UnitOp\n.UnitUse) ("Buffer_Exchange"))
    (Do (UltrafilterID) ('UnitOp\n.UnitID))
    (Do (n+1) (n))
  )
)

(RULE= R49
  (LHS=
    (Is (First_Task) ("Concentration"))
    (> (Product.MW) 10000.0)
  )
  (HYPO= UF_for_Product_Concentration)
  (RHS=
    (CreateObject ('UnitOp\n) (\UNIT_OPS!))
    (Let ('UnitOp\n.UnitName) ("Ultrafilter"))
    (Let ('UnitOp\n.UnitUse) ("Concentration"))
    (Do (UltrafilterID) ('UnitOp\n.UnitID))
    (Do (n+1) (n))
  )
)

(RULE= R48
  (LHS=
    (Yes (Biomass_Removal.Done))
    (> (Product.MW) 10000.0)
  )
  (HYPO= UF_for_Product_Concentration)
  (RHS=
    (CreateObject ('UnitOp\n) (\UNIT_OPS!))
    (Let ('UnitOp\n.UnitName) ("Ultrafilter"))
    (Let ('UnitOp\n.UnitUse) ("Concentration"))
    (Do (UltrafilterID) ('UnitOp\n.UnitID))
    (Do (n+1) (n))
  )
)

(RULE= R47
  (LHS=
    (Yes (WMR_for_Refolding))
  )
  (HYPO= UF_for_Product_Concentration)
  (RHS=
    (CreateObject ('UnitOp\n) (\UNIT_OPS!))
  )
)
(Let ('UnitOp\n\UnitName) ("Ultrafilter"))
(Let ('UnitOp\n\UnitUse) ("Concentration"))
(Do (UltrafilterID) ('UnitOp\n\UnitID))
(Do (n+1) (n))
)

(@RULE= R46
(@LHS=
  (Yes (Polishing_of_Intra_Active_Done))
  (> (Product.MW) (10000.0))
)
(@HYPO= UF_for_Product_Concentration)
(@RHS=
  (CreateObject ('UnitOp\n) (\UNIT_OPS\)
  (Let ('UnitOp\n\UnitName) ("Ultrafilter"))
  (Let ('UnitOp\n\UnitUse) ("Concentration"))
  (Do (UltrafilterID) ('UnitOp\n\UnitID))
  (Do (n+1) (n))
)
)

(@RULE= R50
(@LHS=
  (Is (First_Task) ("Biomass_Recovery"))
  (Yes (Flocculation_Desired))
)
(@HYPO= WMR_for_Flocculation)
(@RHS=
  (CreateObject ('UnitOp\n) (\UNIT_OPS\)
  (Let ('UnitOp\n\UnitName) ("WM_Reactor"))
  (Let ('UnitOp\n\UnitUse) ("Flocculation"))
  (Do (WM_ReactorID) ('UnitOp\n\UnitID))
  (Do (n+1) (n))
)
)

(@RULE= R51
(@LHS=
  (Yes (Buffer_Exchange_DONE))
)
(@HYPO= WMR_for_Refolding)
(@RHS=
  (CreateObject ('UnitOp\n) (\UNIT_OPS\)
  (Let ('UnitOp\n\UnitName) ("WM_Reactor"))
  (Let ('UnitOp\n\UnitUse) ("Refolding"))
  (Do (WM_ReactorID) ('UnitOp\n\UnitID))
  (Do (n+1) (n))
)
)

(@RULE= R52
(@LHS=
  (Yes (Incl_Body_Recovery_DONE))
)

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(HYPO = WMR_for_Solubilization)
(RHS =
    (CreateObject ('UnitOp\n\n\n UNIT_OPS\n))
    (Let ('UnitOp\n\n\n UnitName) ("WM_Reactor"))
    (Let ('UnitOp\n\n\n UnitUse) ("Solubilization"))
    (Do (WM_ReactorID) ('UnitOp\n\n\n UnitID))
    (Do (n+1) (n))
)

(GLOBALS =
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    @INHVALDOWN=TRUE;
    @INHOBJUP=FALSE;
    @INHOBJDOWN=FALSE;
    @INHCLASSUP=FALSE;
    @INHCLASSDOWN=TRUE;
    @INHBREADTH=TRUE;
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    @PWTRUE=TRUE;
    @PWFALSE=TRUE;
    @PNOTKNOWN=TRUE;
    @EXHBWRD=TRUE;
    @PTGATES=TRUE;
    @PFACIONS=TRUE;
    @SOURCESON=TRUE;
    @CACTIONSON=TRUE;
    @SUGLIST=GET_DOWNSTREAM_DATA;
)
APPENDIX III

The Elemental Balance Facility
APPENDIX III.  Macroscopic Reaction Stoichiometry of pST Produced by Recombinant E. coli

The above figure shows a macroscopic view of E. coli cells that produce pST. The cells consume glucose (energy and C source), oxygen, and ammonia (N source) and produce more cells (Biomass), product (pST), carbon dioxide, and water. It should be noted that Biomass in the above picture represents non-pST containing biomass because pST is represented as a separate compound.

Considering the most plentiful components that compose Biomass (C, H, O, N) and assigning a molecular formula to all compounds, one can estimate the stoichiometric coefficients of the overall reaction of pST formation.

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<thead>
<tr>
<th>Component</th>
<th>Molecular Formula</th>
<th>MW</th>
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<tr>
<td>Glucose</td>
<td>CH$_2$O</td>
<td>30.0</td>
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<tr>
<td>Biomass</td>
<td>CH$<em>{1.8}$O$</em>{0.5}$N$_{0.2}$</td>
<td>24.6     (From Roels, 1983)*</td>
</tr>
<tr>
<td>pST</td>
<td>CH$<em>{1.58}$O$</em>{0.34}$N$_{0.29}$</td>
<td>23.08    (From Roels, 1983)*</td>
</tr>
</tbody>
</table>
Overall Reaction:

Glucose + aOxygen + bNH3 --> dBiomass + epST + fCO2 + hH2O

CH2O + aO2 + bNH3 --> dCH1.8O0.5N0.2 + eCH1.58O0.34N0.29 + fCO2 + hH2O

Writing atom balances for C, H, O, and N for the above equation, one gets:

Atom Balance:

\[
\begin{align*}
C: & \quad 1 = d + e + f \\
H: & \quad 2 + 3b = 1.8d + 1.58e + 2h \\
O: & \quad 1 + 2a = 0.5d + 0.34e + 2f + h \\
N: & \quad b = 0.2d + 0.29e
\end{align*}
\]

A matrix representation of the above equations is:

\[
\begin{bmatrix}
0.00 & 0.00 & 1.00 & 1.00 & 1.00 & 1.00 & 0.00 \\
0.00 & -3.00 & 1.80 & 1.58 & 0.00 & 2.00 \\
-2.00 & 0.00 & 0.50 & 0.34 & 2.00 & 1.00 \\
0.00 & -1.00 & 0.20 & 0.29 & 0.00 & 0.00
\end{bmatrix}
\begin{bmatrix}
a \\ b \\ c \\ d \\ e \\ f \\ h
\end{bmatrix}
= \begin{bmatrix} 1 \\ 2 \\ 1 \\ 0 \end{bmatrix}
\]

This is a system of four linear equations with seven unknowns. Therefore, three additional equations are needed to solve the system. Assuming a value for the overall biomass yield (Yx/s), one has:

\[
Yx/s = 0.5 = (24.6d + 23.08e)/30
\]

(III.5)
Assuming that total cell protein constitutes 60% of total dry cell mass and that pST constitutes 20% of total cell protein, one gets an additional equation:

\[ pST = 0.2 \text{ (total cell protein)} = (0.2)(0.6) \text{ (total cell mass) or} \]

\[ 23.08e = (0.2)(0.6)(24.6d + 23.08e) \quad \text{(III.6)} \]

Equations III.1,2,3,4,5, and 6 yield:

\[ a = 0.3544, b = 0.1306, d = 0.54, e = 0.078, f = 0.382, h = 0.6483. \]

BioDesigner is equipped with a facility (see Appendix I) that solves the system of linear equations and assists the user in the estimation of the elemental balance around a reaction system.

APPENDIX IV

DETAILED STREAM REPORT
OF THE pST DESIGN CASE
**APPENDIX IV: STREAM REPORT**  (Cycle Time = 30.0 h)

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<td>BLT101</td>
<td>STR101</td>
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<td>MZX101</td>
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**STREAM PROPERTIES**

| ACTIVITY U/ml | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| TEMP C        | 25.0 | 25.0 | 25.0 | 37.0 | 25.0 |
| PRES BAR      | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

**INTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)**

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**SUBTOTAL**

|       | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

**EXTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)**

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**SUBTOTAL**

|       | 21900.000 | 2451.000 | 24351.000 | 24351.000 | 136.200 |

**TOTAL**

<p>|       | 21900.000 | 2451.000 | 24351.000 | 24351.000 | 136.200 |</p>
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### STREAM PROPERTIES

| Activity  | U/ml | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Temp      | C    | 25.0| 37.0| 37.0| 37.0| 37.0|
| Pres      | BAR  | 1.0 | 6.0 | 3.5 | 3.5 | 2.2 |

### INTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)

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### EXTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)

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**SUBTOTAL**

| 38550.000| 38550.000| 38686.200| 38686.200| 39007.632|

**TOTAL**

<p>| 38550.000| 38550.000| 38686.200| 38686.200| 39007.632|</p>
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**STREAM PROPERTIES**

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**INTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)**

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**SUBTOTAL**

|       | 0.000 | 2752.288| 0.000 | 2752.288| 82.569|

**EXTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)**

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**SUBTOTAL**

|       | 39007.632| 21277.280| 0.000 | 21277.280| 16971.997|

**TOTAL**

<p>|       | 39007.632| 24029.568| 0.000 | 24029.568| 17054.566|</p>
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**STREAM PROPERTIES**

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| Pres      | BAR  | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

**INTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)**

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**SUBTOTAL** 2669.719 0.074 0.000 0.074 0.004 0.000

**EXTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)**

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**SUBTOTAL** 4305.283 6974.928 12000.000 18974.928 15925.816 0.000

**TOTAL** 6975.002 6975.002 12000.000 18975.002 15925.820

-270-
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**STREAM PROPERTIES**

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**INTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)**

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**EXTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)**

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**SUBTOTAL**

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### STREAM PROPERTIES

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### INTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)

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**SUBTOTAL**

|          | 0.000 | 0.062 | 0.000 | 0.062 | 0.000 |

### EXTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)

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**SUBTOTAL**

|          | 5454.900 | 7827.948 | 21000.000 | 28827.948 | 23049.421 |

**TOTAL**

<p>|          | 5454.900 | 7828.010 | 21000.000 | 28828.010 | 23049.421 |</p>
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**SUBTOTAL** | 0.062 | 0.000 | 0.062 | 0.000 | 0.062 |

**EXTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)**

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**SUBTOTAL** | 5778.527 | 375000.000 | 380778.527 | 355378.127 | 25400.400 |

**TOTAL** | 5778.589 | 375000.000 | 380778.589 | 355378.127 | 25400.462 |

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**STREAM PROPERTIES**

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**INTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)**

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**SUBTOTAL**

|          | 0.000 | 0.062 | 0.000 | 0.000 |

**EXTRACELLULAR COMPONENTS FLOWRATE (Kg/Cycle)**

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**SUBTOTAL**

|          | 25276.934 | 123.466 | 5075.056 | 4050.321 |

**TOTAL**

|          | 25276.934 | 123.528 | 5075.056 | 4050.321 |