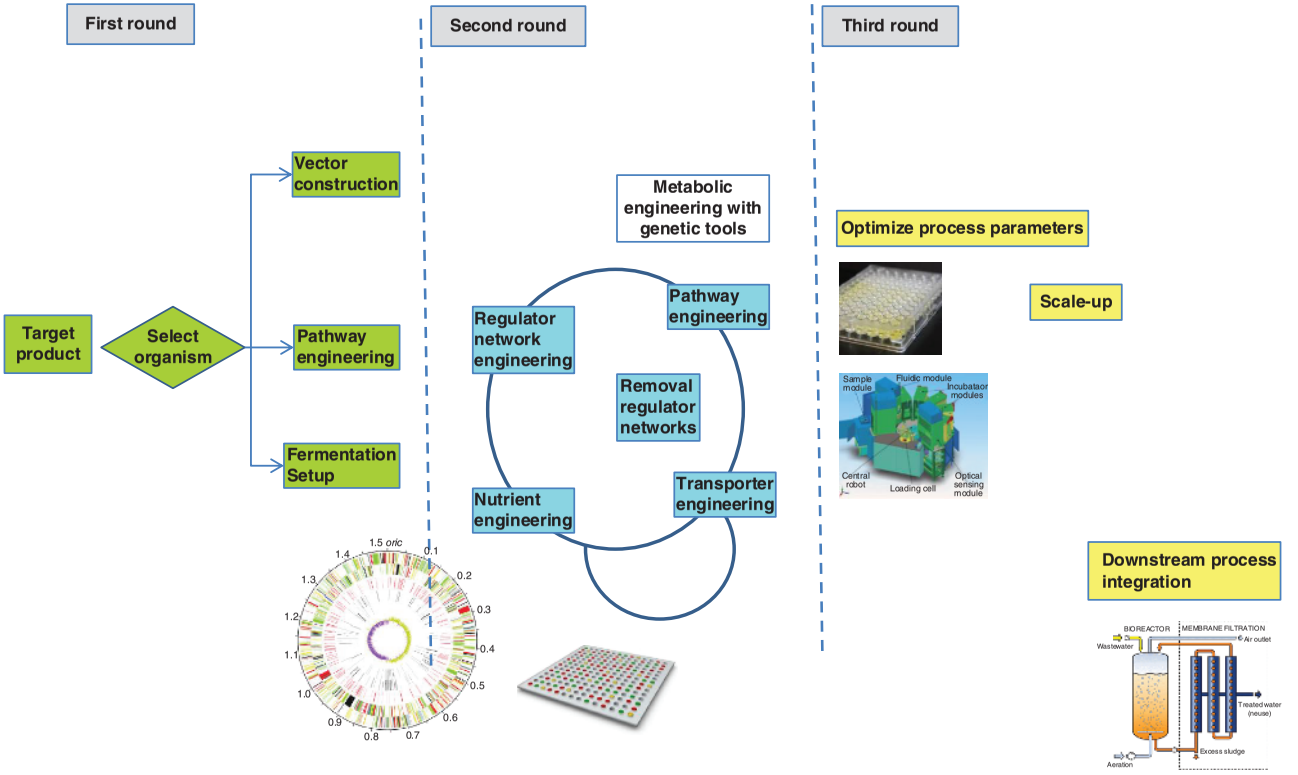

Figure 1.6 A systems biology approach for bioreactor process development based on a three-round procedures.

The base strain is further engineered (ii) based on the results obtained from high-throughput genome-wide data and computational analyses. (iii) The performance of this preliminary production strain is then evaluated in an actual fermentation process. In this step, the downstream processes are also considered. The results are then fed back into further strain development until a superior strain showing desired performance is obtained.

Others put particular focus on the genomic step as being to keystone of successful bioprocessing. Herrgård *et al.* [22], for example, have convincingly shown how yield and expression rates of metabolite and protein products may result in manifold increases by optimal metabolic network identification (OMNI). By this method, potential changes in a metabolic model on genome scale are systematically identified by comparing model predictions of fluxes with experimental measurements. OMNI uses efficient algorithms to search through the space of potential metabolic model structures, thereby identifying bottleneck reactions and their associated genes. The OMNI method has been applied in the optimization of the metabolite production capacity of metabolically engineered strains [23, 24]. Thus, this method could unravel secretion pathways for desired byproducts and suggest ways for improving the strains. By that, a new tool is provided for efficient and flexible refining of metabolic network reconstructions using limited amounts of experimental data – this makes it a complementary resource for bioreactor bioprocess development.

As mentioned earlier, application to mammalian cells tend to dominate new industrial bioprocesses; in consequence, systems biology approaches must be able to deal with models of higher complexity for these cells to provide reliable predictions. The increased complexity of the systems biology task is apparent in the study performed by (Xu *et al.* 2011), where they present a map of the 2.5 GB genomic sequence of the CHO-K1 cell line comprising 24 400 genes located on 21 chromosomes, including genes involved in glycosylation, affecting therapeutic protein quality, and viral susceptibility genes, relevant to cell engineering and regulatory concerns. The huge data collection contributes to explain how expression and growth mechanisms may influence expression patterns related to human glycosylation-associated genes are present in the CHO genome. Again, conceiving systems biology data provide additional cues on the genome level that may facilitate the optimization of biopharmaceutical protein production in bioreactors.

One more key functionality of the biological system is the stability of the genetic material of the cell. The stability of a cloned cell line for recombinant protein expression is an essential function to maintain during a production batch as well as in a cell bank for repeated seeding of cultures. The sensitivity of production cell lines and the implications thereof have been addressed in a variety of studies [26, 27, 28].

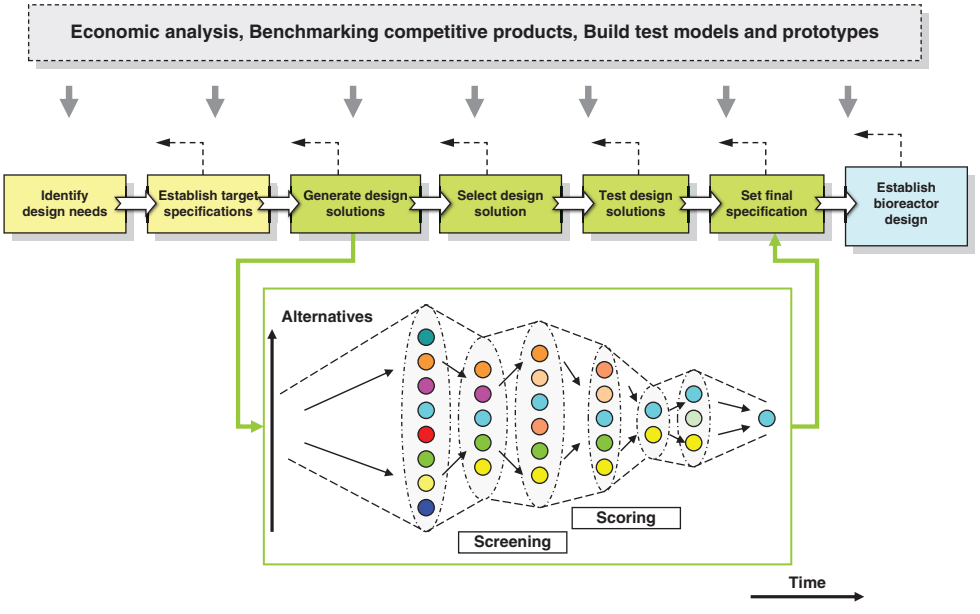

Figure 1.7 Conceptual design principle according to Ulrich and Eppinger sequential design concept where product alternatives are screened versus customer needs.

1.6 Using Conceptual Design Methodology

Another approach that can support bioreactor design and operation substantially is the conceptual design methodology. The basics of the methodology were established in mechanical engineering several decades ago and have since then gradually been refined [29–42]. The main intention was to systemize the design work in a development team in order to reach the best design architecture of a product (Figure 1.7). The approach is a typical top-down procedure: overview all alternative solutions and from that select the best constraints.

Recently, the concept was revived and demonstrated on applications in biotechnology, including bioreactors and bioprocesses [33]. The original methodology was expanded by bringing in the biological systems in the concept and showing how these in the best way could interact with mechanical and electronic systems in the product. Therefore, the methodology was termed *biomechatronics* as it merged complexities from three *per se* complex engineering disciplines: the bioengineering, the mechanics, and the electronics.

A key feature of the biomechatronics methodology is that it is user needs and functionality that guide the design toward the design targets.

Mandenius and Björkman [33, 34, 35] have in a number of examples shown how this can structure and improve the design work for typical biotechnology products and production systems, such as upstream and downstream equipments,

biosensors, biochips, diagnostic devices, and bioprocesses, at the same time as the work process is facilitated and speeded up. [39, 45, 47–49]

Figure 1.8 recapitulates the cornerstones of the methodology: to precisely define and specify the needs and target metrics of the user or customer; to clearly define the expected transformation process (*Trp*) of the product or process and those systems that must interact with that process to carry it out efficiently; to consider all functional elements that must be present for this and to configure (or permute) these in a variety of more or less appealing alternatives; and, finally, to compare and assess these alternatives in order to screening out the ones that best cope with the original design and user targets.

As apparent in the figure, the methodology is based on a consecutive and iterative procedure where graphical and tabular tools support the design work. The flow of work depicted in the figure outlines the recommended steps in a sequential order. In the first step, the design mission is concisely stated. This is followed by identifying the needs of the users of the intended product or production process.

These needs are then further specified with target values. With the help of the specifications, an overview flow chart, the so-called Hubka–Eder map [30], is drawn, which shows the functions and systems required for accomplishing the specification.

The functions in this chart are represented by abstract functional components that are combined in as many realistic alternative permutations as imaginable. This is the key step in the design and is referred to as *concept generation*. The conceptual alternatives are screened and scored toward the original specification target values. This results in a ranking from which the best design alternatives are selected.

First, at this stage, actual physical, chemical, or biological objects are brought into the design work. These objects, the so-called anatomical components, are identified with concrete technical devices, instruments, or other technical gears, usually commercially available, or feasible to construct or prototype. After additional assessment, the anatomical objects form the final design structures of the product.

The conceptual approach is very useful in the design of bioreactors as well as for the layout of the operational procedures of bioreactors and integrated bioprocesses where the bioreactor is a part.

As mentioned earlier, the main purpose of a bioreactor is to control the biological transformations that take place in it.

One way of describing the *TrP* would be to follow the established biochemical engineering approach – to structure the transformation into the biological conversion steps based on metabolic maps and process flow diagrams [36, 37]. This would more or less automatically end up in a description with mass transport and rate constant-based kinetics. This would depend on the environmental state (e.g., temperature- and pressure-dependent constants) and supply of raw materials and media.

In the following example, the systems and subsystems necessary for carrying out the *TrP* in the bioreactor are instead described with the biomechatronic design

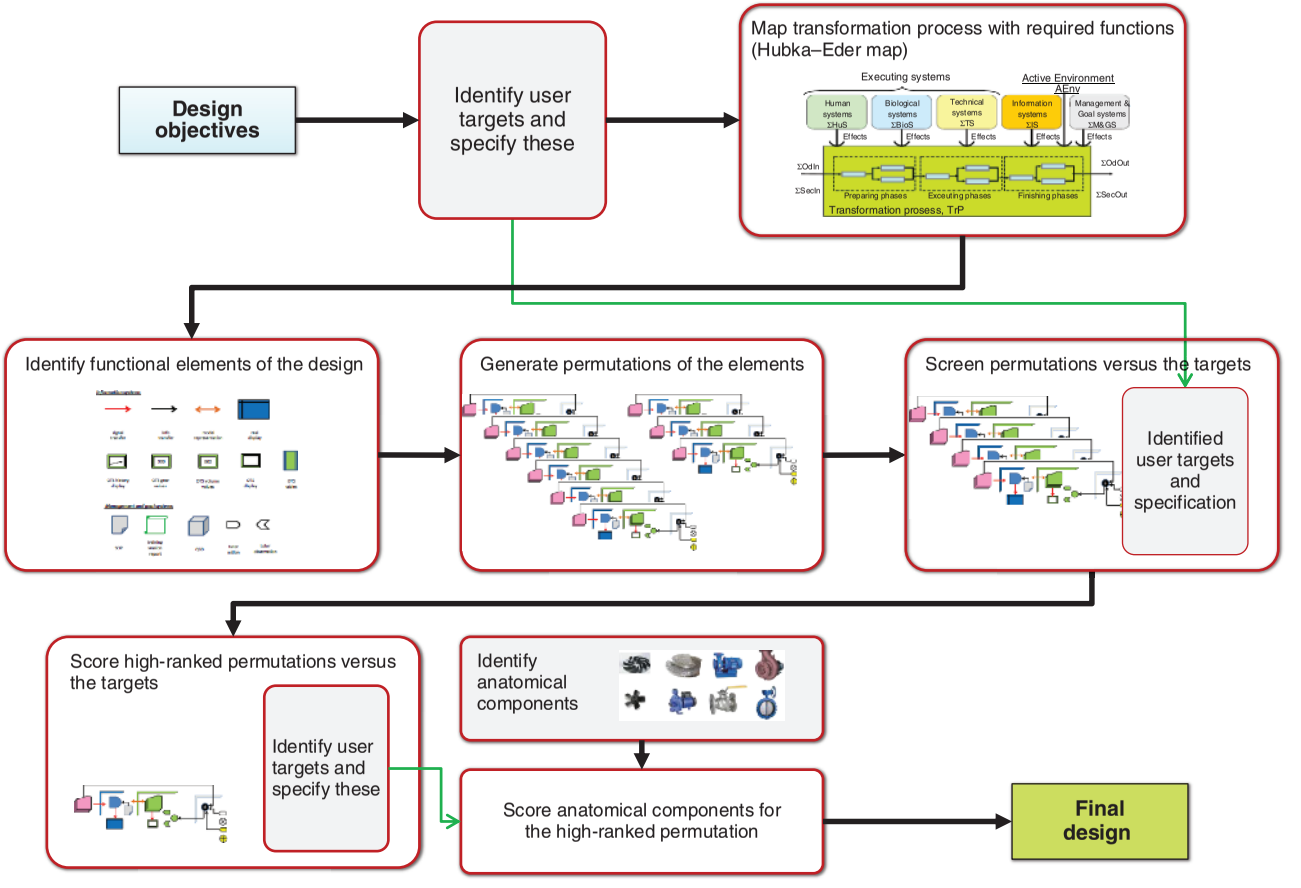

Figure 1.8 The biomechatronic methodology according to Mandenius and Björkman where conceptual methods and tools are combined in a complementary methodology.

approach (Figure 1.9). The *TrP* in the example could be any bioconversion that is possible to realize in a submerged microbial or cell culture system, where nutrients are taken up by the cells and converted into metabolites or protein products. The Hubka–Eder mapping is now used to analyze the interactions between the systems in a generalized way. The biological ($\sum$ BioS) and technical system ($\sum$ TS) entities of the map are here described more thoroughly since these are, of course, pivotal in a bioreactor that performs biological conversions.

Also, as the figure illuminates, the *TrP* of the Hubka–Eder map has an inherent mass balance structure between the inputs and outputs. The map has defined phases (preparing, executing, and finishing), as a conventional process flow diagram has upstream and downstream sections, and in the Hubka–Eder map it is relatively easy to identify those phases where the biologically and kinetically controlled transformations take place. The Hubka–Eder map can be adapted to cover typical bioreactor processes such a recombinant protein expression, viral vector production, or stem cell differentiation.

Figure 1.9 and the zoom-in depiction in Figure 1.10 illustrate a well-known biotechnology application; protein production in a recombinant host cell line is exemplified. The biological systems have in the map been divided into four different biological systems: the culture media system (*BioS-1*), the transport system of the cells (*BioS-2*), the host cell metabolism (*BioS-3*), and the expression system (*BioS-4*). Also, a sub^{3}system and sub^{4}system can be included, preferably using a software tool to support the structuring of the information. It is noteworthy that at higher system levels only functions are described, whereas at the lower levels anatomical structures are introduced, such as a particular nutrient or biomolecules, for example, 30S ribosome and tRNA-amino acid. When the alternative anatomical units are identified the analysis is completed and an anatomical blueprint can be set up (cf. Figure 1.8).

The most essential functions needed in the $\sum$ TS and these functions' interactions with other systems in the Hubka–Eder map of the bioreactor are included in the descriptions shown in Figure 1.10. Here, the $\sum$ TS have been divided in subsystems for the functions of heat exchanging, agitation, pumping (transporting liquids and gases), containment, sterilization (partly overlapping with the previous), chemical state transformers, and pressure generation.

When possible we use the same groups of $\sum$ TS for different bioreactor types. Thus, the *TS-1* system concerns the function of nutrient handling (supply, store, and transport). On the next system level, this will result in pumping or injection, storage containment of nutrients, and means to contact the nutrients with the cells). For example, CO_2 supply through pH balancing of a buffer of gas head space are design alternatives to be ranked toward the cells' transport functions as described in *BioS-2*.

The functions of the *TS-2* system concern containment and agitation. The *TS-2* system should protect the culture from the environment and sometimes the opposite, protect the environment and the $\sum$ TS.

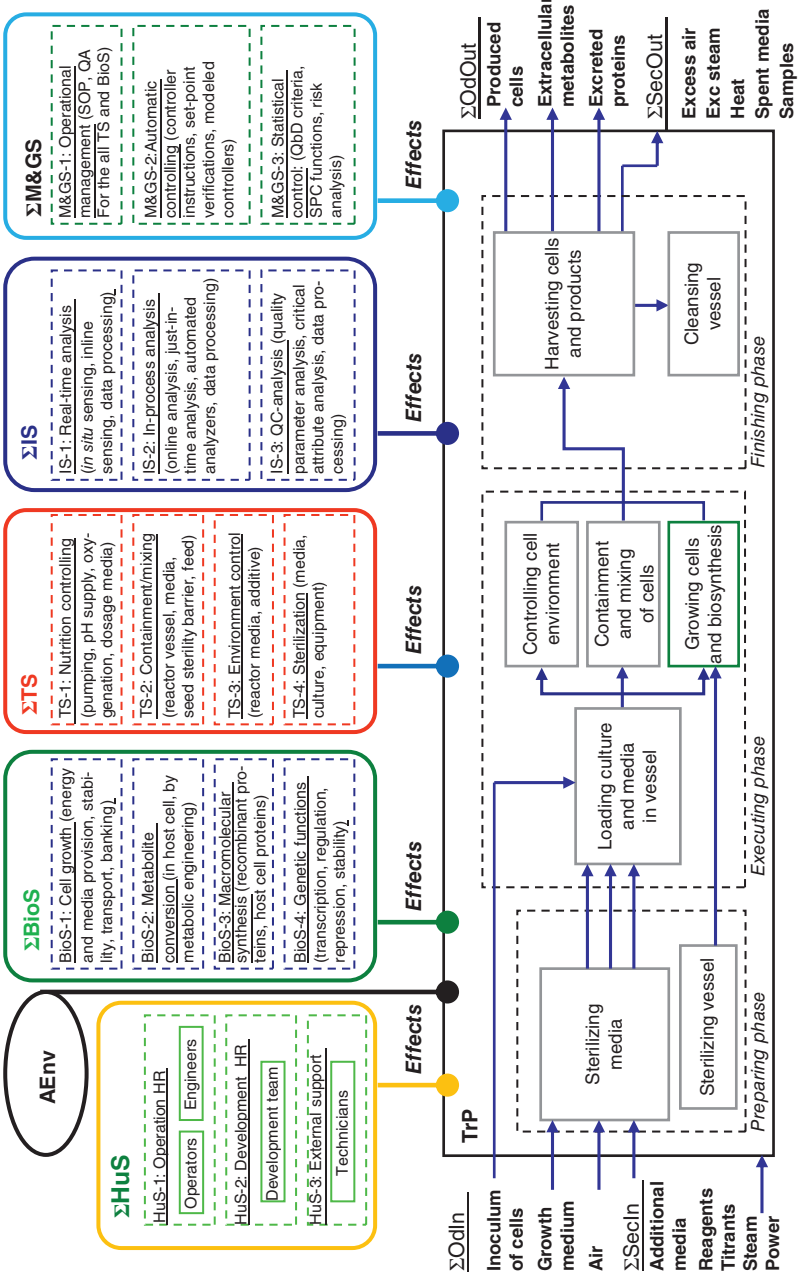

Figure 1.9 Hubka–Eder map of a bioreactor for the microbial production of a recombinant protein. Overall Hubka–Eder map showing the transformation process and the systems and subsystems involved for performing the transformation.

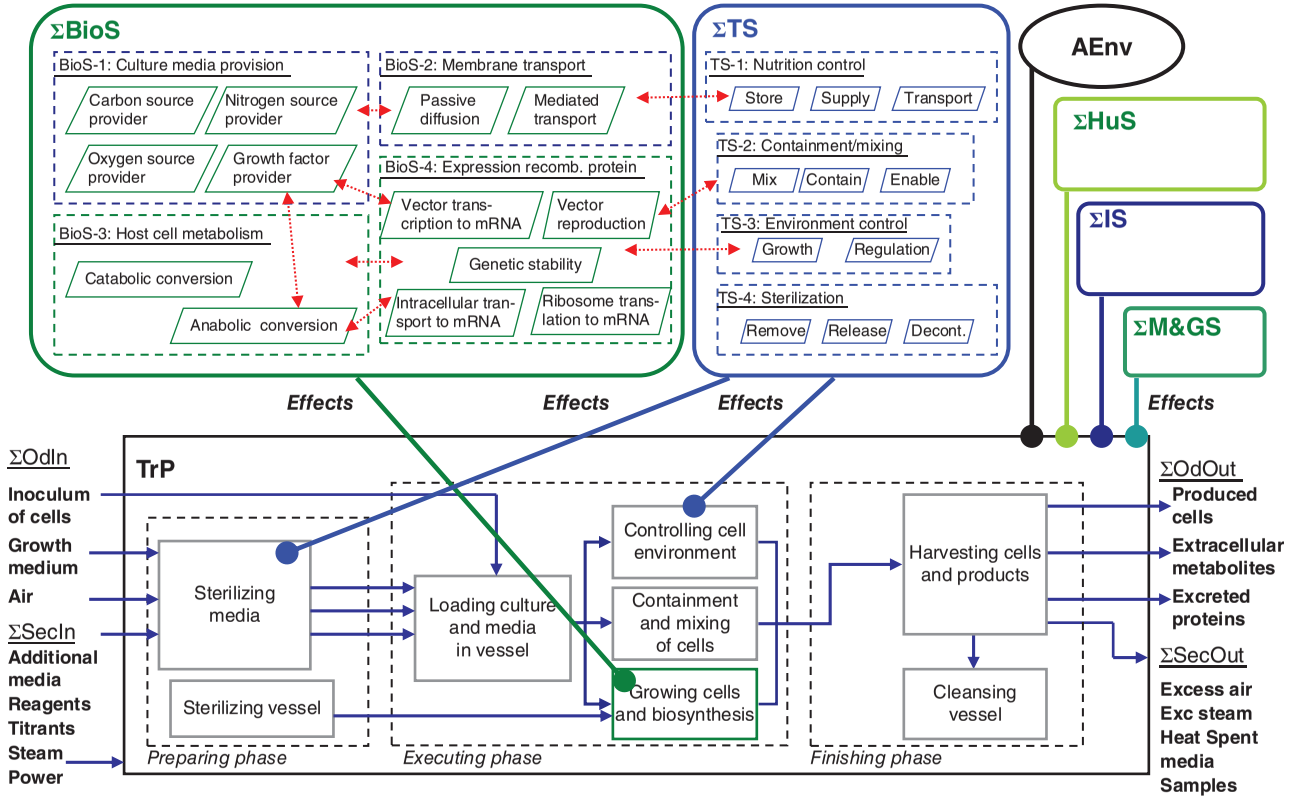

Figure 1.10 A zoom-in of the Hubka–Eder map in Figure 1.9 showing the ∑BioS and ∑TS systems and the interaction of subsystems.

The mixing function is subdivided into agitation of cells, added media, and supplied gas. Anatomical parts for these subsystems could be turbines, draft tubes, and rotating vessels (cf. tissue culture application, discussed later).

Alternative ways of introducing oxygen (e.g., by spargers or silicone tubing) without provoking oxidative or shear stresses on the cells are considered at this stage.

The *TS-3* system provides the functions for control of the bioreactor environment. Subsystems involve heat transfer (e.g., by heat exchanging, pre-heated liquid media, reactor jacket), pH regulation, pO_2 regulation, CO_2 regulation, and pressure regulation and media additives (factors, shear force reducing polymers such as polyethylene glycol).

The function of the *TS-4* system is to provide sterility of the bioreactor. Common operations are *in situ* heating procedures, chemical treatment, and microfiltration. Here, it is also suitable to consider to bring up the less common alternatives such as radiation and toxicant treatment, or to introduce disposable bioreactor vessels that revolve the prerequisites for sterilization procedures significantly.

Table 1.3 resolves the map views of the $\sum$ TS in more detailed subsystems and functions, and gives examples of anatomical components.

For example, the heat exchange subsystem needs a subfunction for the removal of heat (produced by the culture), which could be cooling coils or a jacket. The heat exchanger subsystem also needs a function for heating up the reactor medium, which could be a heat cartridge, hot vapor perfusion, or, again, a heated coil.

Based on the identification and analysis of functional systems in the Hubka–Eder map, critical design elements are conceived and compiled (Figure 1.11a). Here, the most essential elements of the technical and biological systems are shown. Note that it is the functional capacity of the elements that are displayed, to avoid confusing the design work with physical objects at this state but to keep focus on what these objects shall achieve in the design solution.

The functional elements are subsequently combined in order to generate diverse conceptual alternatives (Figure 1.11b). The 12 functional elements are used with very small modifications to envisage combinations that will allow the *TrP* to be realized. The four configurations shown represent just a fraction of all combinations that are possible to generate. Especially, if additional elements were identified and introduced, a variety of other configuration alternatives could easily be generated. This would of course be the case in a large-scale design project (cf. [33]).

The generated alternatives are then screened and scored versus the user needs and specified targets identified in step 1. The total scores for each alternative are used to rank them and to assess which ones are preferable according to the users' targets. This results in a preferred conceptual design for which the functional elements are replaced with real physical objects, the so-called anatomical objects. The four configurations are shown. First, after this conceptual analysis and assessment, the prototyping of the bioreactor ensues. Figure 1.11b could be compared with the 12 bioreactor designs displayed in Figure 1.2.

Table 1.3 The technical systems (∑TS) and subsystems of a typical bioreactor.

Technical system from functionality perspective	Technical subsystems and their functions	Examples of anatomical component for performing functions
Heat transfer system	To keep culture at optimal temperature level	Heat exchangers External loops
	To sterilize the equipment	Steamers
Agitation/mixing system	Disperse air	Sparger, pressure valve Bubbling device
	Mix liquid/air	Turbine impeller Marine impeller Anchor impeller Toroid device Baffles
Transport of media	To transport gaseous media	Pressure vessel Gas flow system
	To transport liquid media	Displacement pump Peristaltic pump Syringe pump/device Flask transfer device Hydrostatic pressure system
Filtration of media	Particle removal Virus removal Heat-labile molecule removal	Mini-filtration Ultrafiltration Microfiltration
Containment	To contain batches repeatedly	Steel vessel Glass vessel Teflon vessel
	To contain one batch	Glass jar Plastic bag
Sterilization of equipment and media	Sterilization of equipment and media together	*In situ* heat sterilization *In situ* chemical treatment *In situ* radiation sterilization Microfiltration
	Gas media sterilization	Microfiltration Flush sterilization
Pressure generation	Headspace pressure generation	Pressure valves/vents
	Air gas generation	In-house supply gas system

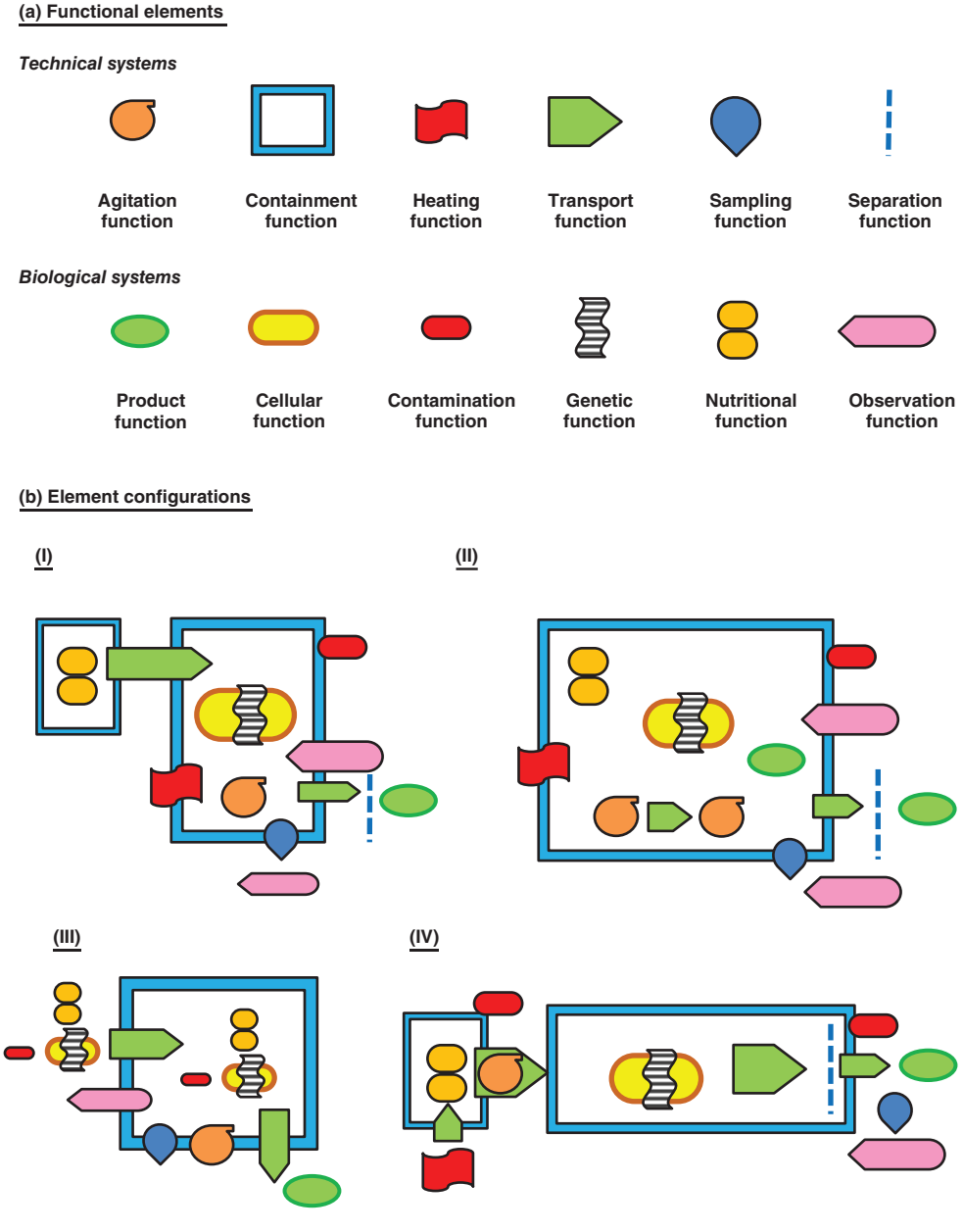

Figure 1.11 (a) Functional elements derived from the systems in the Hubka–Eder map. In a real design, the number of elements may exceed 100. (b) The elements are combined in order to envisage various configurations. The four examples shown can be configured in a variety of other permutations.

1.7 An Outlook on Challenges for Bioreactor Design and Operation

Several of the design issues discussed in this chapter are further evolved in ensuing parts of this book. The challenging nature of the issues diverges. In Table 1.4, the character and potential impacts on bioreactors of these challenges are summarized with reference to where in this book these are further discussed.

Without doubt, a further exploitation of systems biology is one of the potential areas with substantial implications on bioreactors. To continue deriving information about production organisms and their behavior under relevant conditions is, however, a demanding task for future research work. High-throughput analytical machines able to carry out "omics" and data interpretation are currently employed. By that, the bioanalytical systems biology tools may facilitate and improve the conditions of design. The implementation of these data into the bioreactor and bioprocess scenarios versus the production engineering goals requires a synergistic mind-set that is not yet established in the industry. However, there are few reasons to believe that this will not happen in near future.

The combination of the systems biology view with microbial and cellular physiology and how this knowledge is transduced into design practice for more efficient processing is also a challenge required to be further pursued (see Chapter 8).

New biological production systems such as stem cells, tissues, and organs create their own challenges on the design of bioreactors where the intrinsic features and properties of these biological systems require careful consequence analyses for design and implementation (see Chapters 4–6). The early stage of development of these applications may today suffer from not being designed from typical bioengineering aspects, but from a cell biology perspective as suggested in the framework of Figure 1.3.

The use of novel inventive methods of immobilizing cells in order to improve their performance and stability in bioreactors fits well into the increased understanding of physiology of bioproduct-producing cells (see Chapter 7).

Still, traditional biological systems, such as microbial and cell cultures for metabolite and protein production, require the same kind of attention although this has historically been going on for a longer period.

Basic principles and implementation methods for scaling up and scaling out the production systems fit into production encompassing all cell types (see Chapters 4 and 11).

The access to reliable analytical platforms is necessary for good design work; this may include a variety of tools and methods, such as microbioreactors (see Chapter 2), single-use reactors (see Chapter 9), scale-down methods (see Chapter 11), and bioreactors-on-a-chip (see Chapter 3).

Moreover, the technical design of bioreactor equipment has also been supported by other resourceful tools such as DoE for optimization (see Chapter 15), better physical models, computational fluid dynamics, and scaled-down or miniaturized test platforms, which should offer better possibilities (see Chapter 10).

Table 1.4 Challenges of the topics of the book chapters.

Area of challenge	Character and potential of challenges	Chapter in book
Conceptual design	Approach bioreactor design conceptually and systematically; refining the design methodology for a user perspective	Chapter 1
Exploiting systems biology and their tools	The basic principles for bioreactor kinetics, mass, and heat transfer are still applied but are also refined	Chapter 1
The interface between cell physiology and bioreactors	Coping with cellular physiology in the bioreactor applying omics-derived understanding into biological reactions	Chapter 8
Culture of stem cells at bioreactor scale	Adapting bioreactor systems to new cellular production requirements	Chapter 6
	Adapting and scaling up and scaling out bioreactor systems to new cellular systems	Chapter 4
Tissue and organ cell cultures in bioreactors	Adapting bioreactor systems to new cellular production requirements	Chapter 5
Culture immobilized cells in bioreactor	Adapting bioreactor systems to new cellular production requirements	Chapter 7
Down-scaling bioreactor processes	Providing tools representative for large-scale operation at the microbioreactor scale as a process development and optimization tool	Chapter 2
	Providing tools representative for large-scale operation down to microfluidics dimensions	Chapter 3
	Exploiting mass production and parallel process analysis	
Scale up/down methodology	Reducing gaps between scales	Chapter 11
	Reducing gaps between scales	Chapter 10
	Computational fluid dynamics for bioreactor design; understanding rheology of the bioreactor	
Single-use bioreactor design	Facilitating operation by convenience	Chapter 9
Bioprocess integration	Integration of the bioreactor with the downstream process	Chapter 12
Design of growth and production media for bioreactors	Accelerating media optimization by statistical factorial design methods	Chapter 15
Efficient monitoring of bioreactors	Exploiting the information flow from the measurement with modeling Increasing observability by PAT approaches and multivariate data analysis	Chapter 13
	Exploiting models for more information by using soft sensors	Mandenius (Chapter 14)
Training bioreactor operations	Training plant personnel in operating the complexity of bioreactor efficiently	Chapter 16

Although already widely used in bioengineering, it cannot be anticipated that information technology and computer applications will take design and operation of bioreactors several steps further allowing previous studies, methodologies, and existing know-how to be realized in industrial procedures. Examples are applications with multivariate data analysis and process monitoring and control (see Chapter 13) and use of factorial design and optimization of culture media and operation conditions (see Chapter 14).

Radically, new bioreactor designs have been accomplished that replaced old designs in favor of low-cost alternatives that are possible due to novel fabrication methods and materials as well as conditions of cost for operation and materials (see Chapter 9).

Further unfolding of statistics and data mining methods may be foreseen. Other engineering applications, for example, in chemical engineering, are ahead of bioengineering; DoE and related methodologies may be further advanced in the direction of coping with biological variation during extended process periods (see also Chapter 15).

The efforts of bioreactor design cannot be pursued efficiently without the integration of bioreactors into the entire bioprocess. This may essentially generate two gains: better process economics and processes of higher intensity. The implication of this may be huge (see also Chapter 12).

The increasing complexity of integrated bioprocess plants with bioreactors and digital communication requires qualified training procedure. This concerns especially the need for instantaneous decision-making by plant engineers and process operators. In pilot training, rescue training and clinical surgery virtual simulation is applied for accomplishing efficient and cost-effective training of new personnel. There is a challenge to adapt such simulators for bioprocess operator training where in particular variability and unpredictable events in the bioprocesses may be the focus of training (see also Chapter 16).

The CDIO engineering concept [18], referring to that all engineering should preferably be developed along a consecutive process of conceiving (C), designing (D), implementing (I), and operating (O) technical production systems, is indeed applicable to identify design and operation challenges. In Figure 1.12, an update of the earlier CDIO framework (Figure 1.4) is shown where the now-generalized CDIO activities are specified for bioreactor design and operation. The figures emphasize the consecutiveness of design and operation issues. And it provides a map of connectability of the challenges that are elaborately and with details discussed in this book content and placed into the frames of CDIO concept. However, it also reveals some gaps that need to be bridged by novel contributions.

So far, most of these progressing activities are still in the academic research environment. In a few cases, they emerge as new products from Small and Medium-sized Enterprises (SMEs).

Others are already in regular use at the process research and development units, especially at larger biotech companies.

Generation of knowledge and inventions may sometimes thrive best in the academic research supported by public resources, while sometimes it may best be

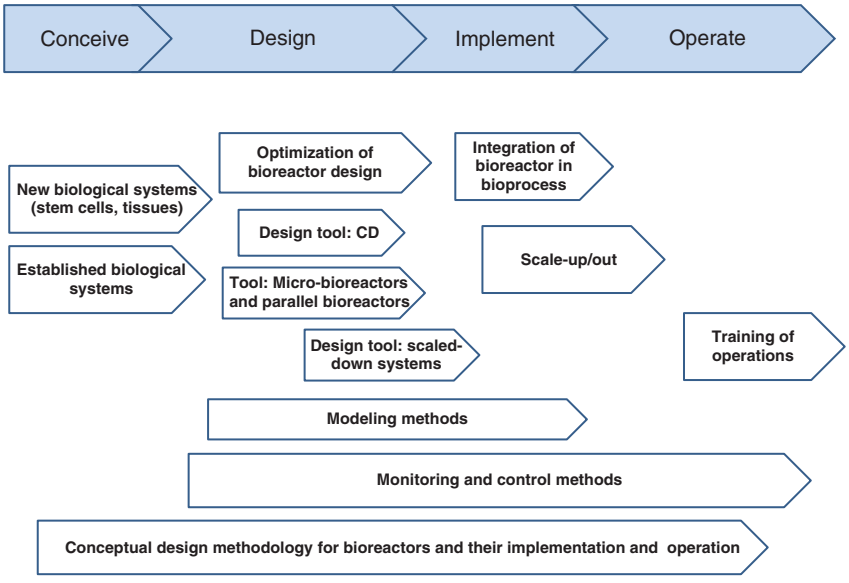

Figure 1.12 The CDIO concept as defined in Figure 1.4, here adapted to bioreactor design and operation with several of the topics and challenges addressed in this book.

developed in-house by companies close to the applications and under knowledge protection.

This book, hopefully, contributes to overview the needs and possibilities and stimulate further progressing.

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