1
Challenges for Bioreactor Design and Operation

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1.1
Introduction

As per definition, the bioreactor is the designed space where biological reactions take place. Hence, the bioreactor is essentially an engineering achievement and its design a challenge for bioengineers.

The bioreactor should create a biosphere that as profoundly and adequately as possible provides the ideal environment for the biological reaction.

The path for reaching, attaining, and maintaining this is the main task for bioreactor engineers to find. That task decomposes into several endeavors necessary to accomplish. One is to design the physical entity of the bioreactor itself – by that, ensuring favorable physical conditions for transport of gases and liquids and solids over time. Another is to ensure that the physical entity of the bioreactor is favorably adapted to the biological system that performs the bioreactions. Yet another is to ensure that the dynamic biophysical and biochemical events taking place are operable in an industrial environment.

In some of these design perspectives, bioreactor design is addressed at a process development stage where the performance of operations is independent of scale or biological system inside the bioreactor. Others address specific biological systems and the particular requirements of these. Others take the viewpoint at the holistic level: how to integrate the bioreactor and its design into an entire bioprocess with the constraints that this creates. Others concern provision of methodologies for observing the bioreactor at R&D as well as at operation stages in order to monitor and control and to optimize its performance from a variety of needs and purposes. Others provide better methods for supporting plant engineers and technicians to manage to operate the bioreactor processes under unpredictable industrial conditions where unexpected events, faults, and mishaps must be interpreted in short time and acted upon.

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Importantly, all these aspects on design and operation may, and even must, be amalgamated into coherent design methodologies that are conceivable and practically achievable. It is the ambition of this book to provide a collection of design options where engineering principles and design tools are presented that facilitate to develop and apply good solutions to emerging needs in bioreactor design.

1.2 Biotechnology Milestones with Implications on Bioreactor Design

The bioreactor is a historical apparatus known since ancient times. Old antique cultures were able to solve bioengineering design challenges for practical purposes such as wine and beer making from mere experience and observations. This paved the way for the evolvement of biotechnological processes, primarily for preparation and production of food products [1].

The notion that microscopic life is a huge industrial resource came gradually to man and with some resistance from the established scientific society itself. An array of fundamental scientific steps paved the way for the unfolding of industrial biotechnology. Growing understanding of the mechanisms of diseases and its interplay with cell biology supported the development.

In the early nineteenth century, scientists such as Lorenz Oken (1779–1851), Theodor Schwann (1810–1882), and others did stepwise begin to fathom the fundamental principles of the cell's behavior in the body and in culture [2]. Louis Pasteur (1822–1895) took these observations and conclusions further into a coherent description of the fermentation mechanisms [3]. Later, researchers such as Emile Roux (1853–1933) and Robert Koch (1843–1910) realized the implications to bacteriology and for spread of diseases. These consorted ascents in cell biology and medicine did synergistically create the necessary background for the exploitation of the industrial potential of cells. By that, also important prerequisites for a furthering of bioreactor design were set.

The microbiology research brought better insights into the up-till-then-hidden processes of the cell and, hence, to the development of bioengineering and to the widespread industrial biotechnology applications during the twentieth century. It is in this framework of bioindustrial activity and progress the bioreactors and their design have been shaped. Still, it is noteworthy that 100 years ago an industrial bioreactor facility did not look too different from today's industrial sites (Figure 1.1).

In the early twentieth century, large-scale fermentation processes were set up with impact onto the war-time industry of that period. Glycerol production for use in the manufacture of explosives, using yeast for conversion from glucose, was established. Another contemporary example is the large-scale production of butanol and acetone by butyric acid bacteria, as developed by Chaim Weizmann, used first for explosives and then for rubber manufacture in the emerging car industry [4]. However, these bioprocesses were soon abandoned for petroleum-based products that had better process economy.

(a)

(b)

Figure 1.1 (a) An old fermentation plant from the late nineteenth century. (b) A modern fermentation plant one century later. The gap in time between the plants reveals that some of the design features have undergone changes, while others are unchanged: the bioreactors are cylindrical vessels, the containment of the broth and concern about contamination were in former days less, piping are essential, many vessels are using the available plant space, and few plant operators are close to the process.

The story of the development of antibiotics is an impressive example of how microbiology and industrial biotechnology evolved over an extended period of time by consorted actions between academic research and industrial product development. The original discovery in 1929 by Alexander Fleming of the antibiotic effect of a *Penicillium* culture was in a series of steps for amplifying the yield and activity of cultures transferred into large-scale production [5]. And other renowned scientists such as Howard Florey, Ernest Chain, Norman Heatley, Marvin Johnson, and others in close collaboration with pharmaceutical companies managed to identify, stabilize, exploit, select strains, exploit genetics, mutational methods and, finally, establish large-scale bioproduction in bioreactors for meeting global medical needs for curing infections [6]. The latter did indeed challenge the engineering skills in understanding optimization in the design and operation of the bioreactor. It also gave ample examples of how knowledge and skills from one group of products could be transferred into others and, by that, pave way for other antibiotics such as cephalosporins, streptomycins, and aminoglycosides.

These endeavors and experiences contributed substantially to facilitate forthcoming bioprocess development of biotherapeutics. Undoubtedly, the concept of process intensification was driving the development although the term was not yet coined. The same was true for the transfer of the concept of continuous strain improvement of microbial strains and cell lines.

In parallel with the progress of developing antibiotics, other microbial primary and secondary products were realized. These included amino acids (e.g., glutamate and lysine) and organic acids (e.g., vitamins) used as food ingredients and commodity chemicals and reached considerable production volumes. Microbial polymers such as xanthan and polyhydroxyalkanoates are other examples of bioprocess unfolding during the mid-1950s [7].

Protein manufacture, especially industrial enzymes, became comparatively soon a part of the industrial biotechnology with large-scale production sites at a few specialized companies (e.g., Novo, Genencor, Tanabe). At these up-scaled processes, very important findings and experiences were reached concerning bioreactor design and operation. Although not yet exploiting gene transfer between species for these proteins, significant technology development for later use was accomplished [1].

Subsequently, the emerging industrial use of animal cells came about. Culturing at large scale, at lower cell densities than fungi and yeasts, and with much lower product titers posed a next challenge to bioreactor engineering [8].

With the ascents of Köhler and Milstein (1975) in expressing monoclonal antibodies in hybridoma cell culture and the ensuing setup of cell culture reactor systems for production, a new epoch came across which has impacted industrial biotechnology and bioengineering tremendously. It initiated a art of cultivation technology where conditions and procedures for the operation of a cell culture showed a number of constraints necessary to surpassed in order to make processing industrially feasible [10].

However, it was the genetic engineering and recombinant DNA technology that created a revolution in the field of industrial biotechnology with macromolecular products from cells, first in bacteria and yeast and subsequently in animal and human cells [11]. Industry was proactive and efficient in transforming science into business activity. In California, Cetus and Genentech were established in the early 1970s. In the years thereafter, Biogen, Amgen, Chiron, and Genzyme followed, all with successful biotherapeutic products in their pipelines – insulin, erythropoietin, interferons, growth hormones, blood coagulation factors, interleukins, and others reached the therapeutic market with relatively short development times, in spite of regulatory requirements and the multitude of novel production conditions spanning from clinical considerations to new manufacturing methodology. Especially, the latter embodied numerous challenges for bioprocess and bioreactor engineering to disentangle.

The latest steps in bioreactor engineering are related to cell production and applications with regenerative medicine products and pluripotent stem cells [12]. Certainly, this has had implication on bioreactor design in terms of new and diverse requirements of performing cellular transformation including cell differentiation, expansion, and maturation, and of longer process time compared with previous processing. The controllability demands of bioreactors for these purposes are higher due to more vulnerable cell types, more complicated growth behavior, and substantially different operations. This addresses again the critical issues of mass transfer and barriers of oxygen, nutrients, and sterility of the cultures.

Table 1.1 recapitulates the milestones of this industrial biotechnology evolution based on the events of modern biology and life science.

Furthermore, the industrial biotechnology development during the twentieth and early twenty-first centuries has been profoundly interconnected with a variety of specific challenges within biochemical engineering research [13]. These have, for example, regarded such issues as the lack of robustness of enzymes and microbial strains as compared with heterogeneous catalysts used in the chemical industry; the difficulties of redesigning cells and other biocatalysts with metabolic pathways adapted to the production of specific products; and challenges in the fermentation of complex raw material streams such as hydrolysates from lignocelluloses or other renewable resources. This has been an integral part of the development where bioreactors have been one of the enabling tools.

Solutions to these challenges are many: using combinations of biological and chemical reactions, genetically engineered crops with better properties for the actual production process, and systems biology methodologies applied on the production microorganisms or cells in order to engineer pathways for metabolic conversion, transcription, and expression.

The academic contributions to this research have been substantial and have led to important industrial improvements such as reduction in process development times and pretreatment, and the ability to handle renewable raw materials applicable to a wide variety of bioprocesses including large-scale biorefinery and waste treatment plants.

Table 1.1 Milestones in industrial biotechnology.

Major achievement	Discoverers	Year	Impact on industry applications	Implication on bioreactor design
Understanding of fermentation principles	L. Pasteur	1857	Initiating wider application of fermentation in industry	The needs for efficiency of design recognized
Anthrax bacteria	R. Koch	1876	Disease effect of bacteria and the uniqueness of a specific bacterium	
Use of antiseptics realized	I. Semmelweis	1846	Chemical control of infections	The success of large-scale microbial production and its dependency of sterility
The existence of biological catalysis	M. Traube	1877	The catalytic action of microorganisms	The optimization of the biocatalytic activity in a bioreactor
Glycerol from yeast cultures	Neuberg *et al.*	1914–1918	Need for glycerol in the war industry	Cultivation of yeast for other products than beer and wine
Acetone–butanol fermentation	C. Weizmann	1914–1918	Supply of bulk chemicals for explosives and car tires	Scale-up technology challenged to meet market demand
Penicillin discovered	A. Fleming	1929	Pharmaceutical biotechnology initiated	Strain improvement
Penicillin isolation	H.W. Florey and colleagues	1939	Product characterization	Yield improvements
Cephalosporin fermentation	Brotzu and Abraham	1948	Other microbial metabolites could act as antibiotics	Fed-batch operations
Antibiotic strain improvement	S. Waksman and others	1940s–1950s	Higher yield per volume	Process intensification

Amino acid fermentation	Kyowa Hakko Co.	1957	Metabolism in strains for amino acids is exploitable	Scale-up of microbial fermentations
Organic acid fermentation	Food industries	1940		Large-scale fermenters
Vitamin fermentation	A. Guilliermond Reichstein	1930s	Riboflavin (B2) (vitamin C)	Bioreactor processes including semisynthetic steps
Genetic engineering and recombinant DNA technique	P. Berg, D. Glaser	1971	Improved metabolism and expression in cells	Induction procedures in bioreactor
Recombinant insulin, growth hormone	H. Boyer and R. Swanson, Genentech	1978	The recombinant DNA-technique open for a biotherapeutic production	A new methodology of culturing recombinant microorganisms with induction protocols
Monoclonal antibodies in hybridoma cultures	G. Köhler, C. Milstein	1975	Diagnostics and therapeutics based on antibodies	Bioreactors to be developed for cell culture requirements, in particular, hybridoma
Recombinant DNA technique applied industrially in animal cell cultures	Pharmaceutical industries	1990s	Recombinant products in mammalian cells human-like biotherapeutics (e.g., EPO, tPA, IFN, Factor VIII)	Bioreactors to be developed for other cell cultures such as CHO, HEK, and other cell lines
Pluripotent stem cells and derived cells	S. Yamanaka	2006	Cells from hES and iPS cells the potential to become new products for cell therapy	Bioreactors must be adapted to new cultivation conditions

Also, the education and training of new bioengineers have evolved a thinking of biochemical engineering that has impacted industry with new perspectives where the biology scope was merged into the engineering framework of conceiving, designing, and operating industrial processes. It is, here, relevant to remind about the tremendous increases in biological knowledge with related technologies that have emerged, which must be integrated and constantly updated into bioengineering science.

1.3 General Features of Bioreactor Design

As Table 1.1 demonstrates, the ascents of the twentieth-century biotechnology have created a pull for advancing bioreactor design. Especially on large scales, the requirements of the cultivation system have dispersed into a variety of diverse technical issues that most of them have in common transfer of mass and energy [14]. In textbooks, a bioreactor is typically described as an apparatus shaped like a chamber for growing organisms such as bacteria or yeasts that can be used for the production of biomolecular metabolites or biopolymers or for the conversion of organic wastes. This very general bioreactor description clearly highlights the main purpose of the design efforts: to accomplish conditions where diverse cell types are able to grow efficiently and produce a variety of biological products with a wide range of molecular sizes in a single unit. This calls for profound adaption of the technical design of the bioreactor system and could expectedly result in many different design solutions. The diversity of the design mainly caused by the time factor; due to the fact that rates differ largely from one organism to another, in reproduction rates, in rates of molecular processing in the individual organisms, and transfer across biological barriers of the cellular systems.

The time factor also applies to the operational procedures. When cells grow, the design must adapt to compensate for the magnification of the dynamics due to higher cell numbers. This mostly concerns supply of nutrients and growth factors. However, it may also be about removal of mass and energy to avoid overloading the system with any of these. The operational procedures shall in combination with the design effectuate this.

A variety of conditions, operational procedures, and considerations are critical for the efficiency of the design (Table 1.2). Transfer rates of mass and energy are among the most critical issues [15].

Environmental factors in wider sense should be considered, as well as ambient temperature and moisture and occurrence of contaminants; all examples of factors may play a major role.

Sterilization is an operational procedure that differs only slightly depending on the organism but must be carefully adapted to the bioreactors' geometrical shape and construction materials. The prevalence of single-use units made in plastic materials highlights the actuality of this issue.

Table 1.2 Bioreactor design criteria.

Design issue	Purpose	Design means	Parameters
Gas transfer in submerged culture	Ensure high growth rate, avoiding oxygen starvation	Reactor geometry Sparger design Baffles Overpressure Impeller geometry	Aspect ratios $K_L a$ *OTR* *OUR* *CER*
Mixing efficiency	Avoiding gradients of heat, nutrients and additives, stress Reduce power	Impeller geometry Baffles Mixing analysis CFD	Aspect ratios Mixing time t Power number
Nutrient supply and addition	Efficient transfer to bioreactor volume	Feeding regime Multiple ports	Linear and exponential profile
Liquid–solid transfer	Enhance reaction rate Reduce gradients	Flow distributors Porous support	Thiele modulus
Heat transfer	Efficient removal of metabolic heat	Internal coils Recycling of media Jacket Cooling media	Dimensionless numbers
Sterility	Ensure whole unit is devoid of foreign microorganisms to avoid infection	Sterilization procedure Overpressure Barriers Containment Microfilters	Sterilization time and temperature
Strain selection	Finding strain with properties adapted to media and reactor constraints	Microbial analysis Omics	Specific rates (μ, q_P, q_S) Inhibition constants
Scale-up procedure	Ensuring same conditions at large scale	Design geometry of vessels and impellers Range of mixing	Aspect ratio Scale-up rule parameters Dimensionless numbers
Rheology		Additives affecting viscosity CFD	Reynold's number CFD data
Homogeneity of culture	Avoiding gradients for ideal reactor conditions	CFD	Zonal analysis data
Media composition	Balanced culture media	Factorial analysis Omics methods	Model fit parameters

Inoculation of cells is another consideration that relates to the size of the inoculum, the state of the cells to enter an exponential growth phase, their variability and sensitivity to microenvironmental conditions, and their purity.

Media composition is an example of yet another design issue comprising both the chemical aspects related to the nutritional value of the media as well as the biological significance of the components in relation to metabolic pathways involved in the growth and production of the cells.

Kinetic relationships in the bioreactor are described for a multitude of state and conditions. Table 1.2 summarizes most essential conditions and key parameters and how they influence design work and options. The access to these key parameters helps in understanding the frames of bioreactor design and why different design alternatives are needed as extensively discussed by many authors [16].

Based on these premises, a diversity of bioreactor design alternatives are have emerged (Figure 1.2). The stirred tank bioreactor is, with few exceptions, the predominantly used design for submerged cultures due to its versatility, operability, ability to cope with many of the aforementioned requirements, and manufacturability (Figure 1.2a).

The main shortcoming of the stirred bioreactor, its mechanical agitation, is solved in other bioreactor designs. In the bubble column bioreactor, the mechanical impeller is exchanged with raising bubbles, which, in the case of an aerobic fermentation will anyhow be required and to the benefit of fewer mechanical components and need for lesser electrical power (Figure 1.2b). But with a sometimes critical drawback, lower volumetric oxygen transfer, which may be a severe shortcoming for fast-growing organisms and high-density cultures.

The airlift bioreactor with a forced flow in an internal or external loop has the same advantage, although the design requires an addtional construction part, the down-comer tube (Figure 1.2c and d). These relatively minor design modifications appear to substantially limit the widespread use of these bioreactor types. Also, the fluidized bed reactor where cells are recycled by external pumping and soluble product harvested by overflow provides the same pros and cons, lesser mechanics, and lower oxygen transfer (Figure 1.2e). However, a density diversity between media and cells is needed, which makes aggregating cells such as flocculating yeast cells or possibly immobilized cells the ideal state for the biological component.

By that, the tank reactor design approaches the tubular reactor designs and solid-state fermentations. In the trickle-bed bioreactor, cells are grafted to a solid material while the medium is fluxed through a bed of biocatalyst (Figure 1.2f). This resembles the chemical engineering tubular reactor model where the catalyst is typically a transition metal catalyst. In contrast to the chemical reaction systems, the biocatalysed reactors harbour low-temperature aerobic processes with profoundly deviating kinetic regimes.

The solid-state bioreactor design can also follow the ancient Chinese tray reactor model as applied in *koji* fermentation. Collecting the culture on a support material, a kind of immobilization procedure, in trays placed in a container with controlled conditions is not optimal but convenient and well proven (Figure 1.2g). The tray bioreactor can be transformed into a static bed reactor, which provides

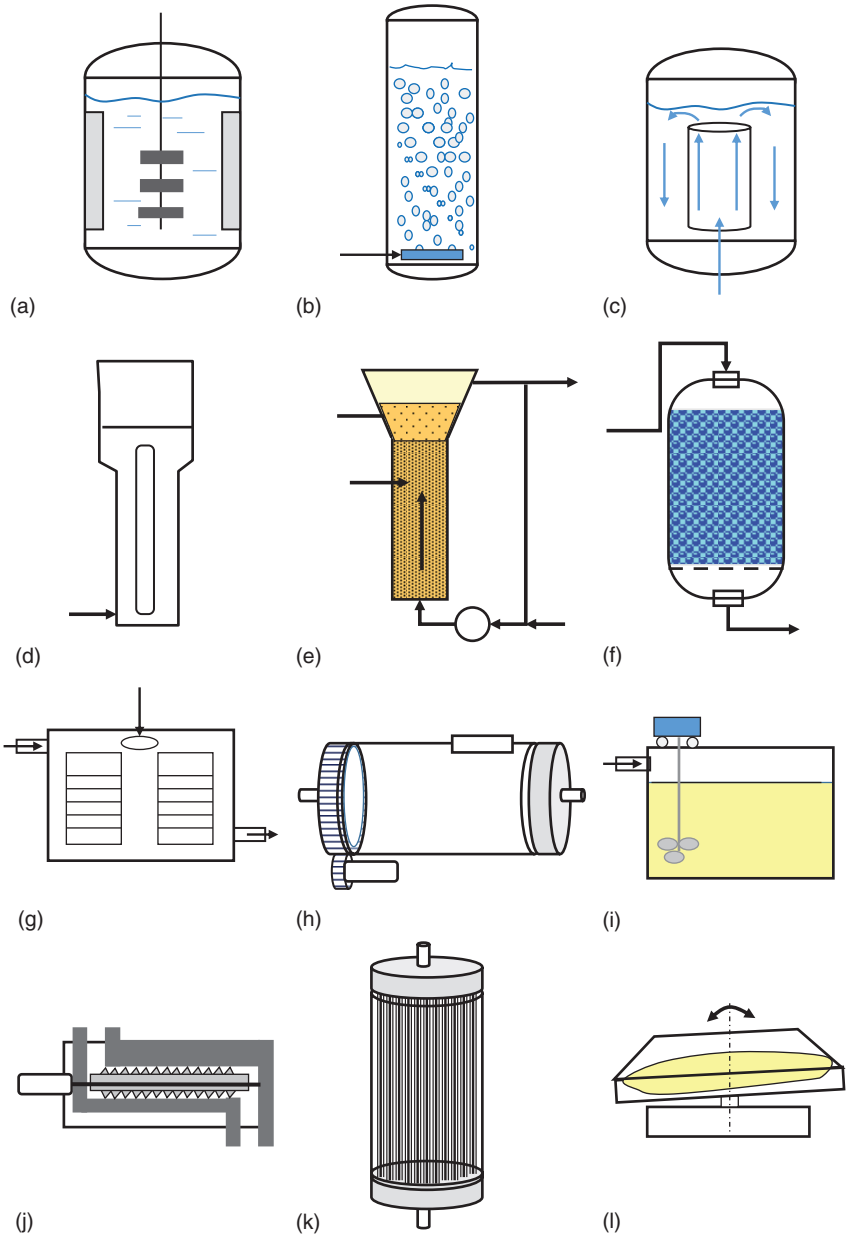

Figure 1.2 Twelve examples of bioreactor designs: (a) stirred-tank reactor, (b) bubble reactor, (c) airlift reactor, (d) loop reactor, (e) reactor with immobilized cells, (f) fluidized reactor with recycling of cells, (g) solid-phase tray reactor, (h) rotary drum bioreactor, (i) agitated-tank reactor with movable impeller, (j) continuous screw bioreactor, (k) hollow-fiber reactor, and (l) wave bioreactor

better efficiency in contacting the reaction components in a flow-through manner. The static bed is a short version of a tunnel bioreactor that allows more catalyst to be contained at equivalent flow rate.

Rotating the bioreactor is another way to agitate the cells and reactants, where the gravity of the particles must be employed to cause the movement in the fluidium, either in a disk or a drum geometry (Figure 1.2h), or by moving a plastic bag (Figure 1.2i). Another technical solution is to move the impeller around in the bioreactor – back to where we started (Figure 1.2j). The design can of course here vary substantially. In waste water plant, for example, the reactor is a wide but low circular tank with the impeller on a rotating arm. As the example suggests, the approach is mostly a solution for the large scale. A continuous screw is a third alternative for creating movement in the bioreaction system, which is most appropriate if the liquid phase to be moved forward is viscous and where the energy input for driving the screw is negligible (Figure 1.2j). Cell cultivation conditions have generated a few additional design forms such as hollow-fiber reactors (Figure 1.2k) and wave bioreactors (Figure 1.2l).

The bioreactor designs quite often need to consider the forthcoming downstream steps. The volumetric rates and equipment size should cope in a realistic way. Often, a larger number of parallel units must be connected to a single reactor if the convenient operational volume of the subsequent step is too small to harbor the volumetric flow. However, the opposite can also apply; the capacity of a single high-speed centrifuge can suffice in a brewery with several fermenters.

1.4 Recent Trends in Designing and Operating Bioreactors

The basic principles of bioreactor design outlined in the previous section have in more recent years unfolded into more elaborate methods due to several new premises. One is the access to computers for complex calculations. This has allowed computations previously too heavy and demanding to perform and too inconceivable to predict. By that, design work can be carried out in much reduced time and with much lesser brain efforts, in order to elucidate metabolic networks, fluid dynamics, and more complex kinetic models of the cell. However, the interaction between this newly created extended knowledge and tools and the problems that require solving have so far only been marginally exploited. Another is the manufacturing cost premises for equipment. Mass production of disposable units has become considerably less costly using new durable plastic construction materials and automated assembly of bioreactor equipment.

The sway from traditional bioreactors of the diverse designs as mentioned in the previous section to single-use reactors has changed the mind-set in the planning for plant engineering concerning estimates of equipment investments and operational costs. The emergence of the wave bioreactors with disposable bioreactor bags in sizes from 1 up to 2000 l took surprisingly long time despite the obvious

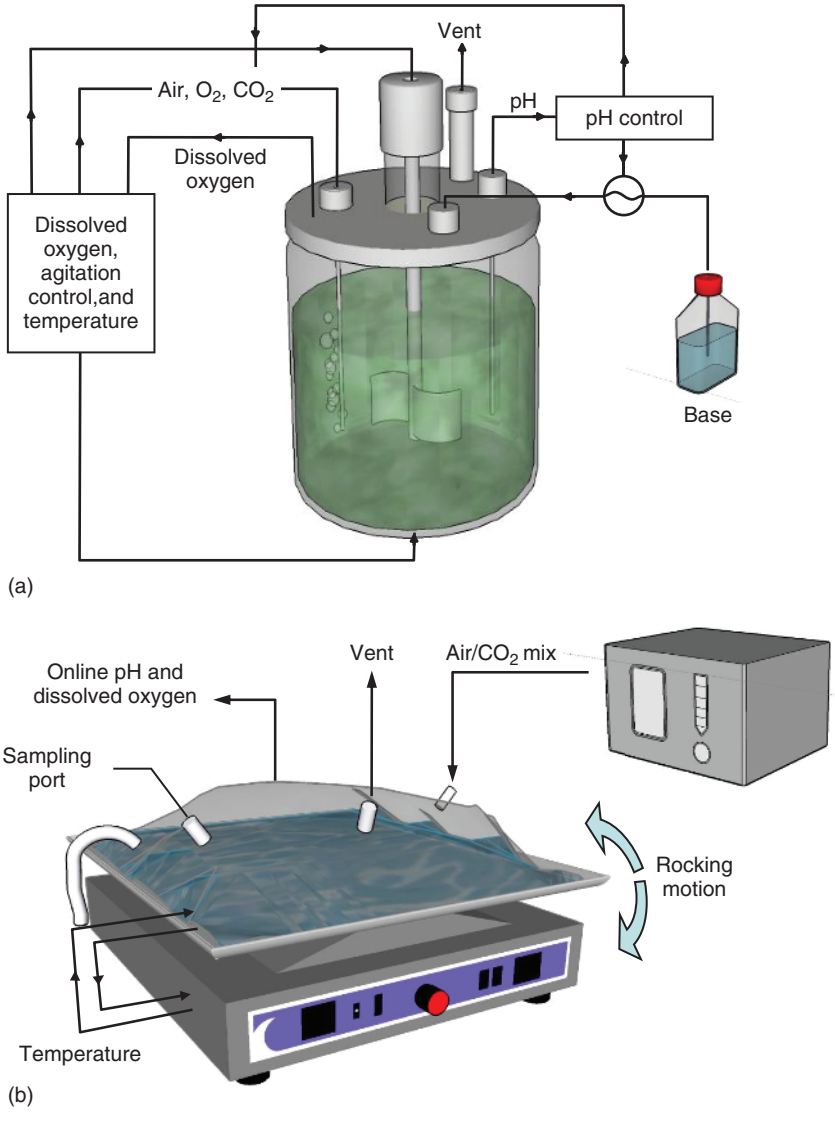

Figure 1.3 An emerging new trend is the replacement of old stainless steel fermenter with single-use wave-bioreactors. It is a striking example of how smart designs based on fabrication technology use compatible low-cost materials and new conceptual thinking lead to a leap in design (from [17], with permission).

advantages they furnish for all involved – the operators, user-companies, and the supplies of units (Figure 1.3).

In principle, the same kind of progress in manufacturing technique, the capability of low-cost mass-production of polymeric materials, allows microfabrication

of small devices, such as microbioreactors and microbiochips. This has in particular imposed implications on R&D processes by facilitating parallel testing of strains, cell lines, and media as well as basic cultivation parameters. Optimization of the bioreactor and the bioprocess can, using these tools, be accelerated significantly. The access to more compact and stable electronics, also due to the availability of mass-production and miniaturized fabrication techniques in the electronic and software industry, facilitates transfer of signals in the microelectronic environment of instrumentation of bioreactors. The capabilities of software programs and implementation of data processing have advanced considerably and could be anticipated to be substantially improved.

Better and more efficient education principles of bioengineers empower the biotech industry with the opportunity to improve process design and development. The conceive–design–implement–operate (CDIO) concept, founded at MIT in Boston and based on a revitalization of traditional engineering training and education with a new conceptual approach, also covers the key elements of bioreactor design and operations [18, 19]. The CDIO concept sees engineering work as a consecutive mental and practical process where ideas are formed and conceived due to eminence of understanding and analytical thinking, where these notions are applied in the design stage and further turned into implementation of systems, plants, and devices that are delivered for operational and perpetual usage (Figure 1.4). Although the CDIO concept is an initiative intended primarily for engineering education, it inevitably has the potential to influence industrial mindsets and work in a positive sense. If the CDIO principle is unanimously accepted and applied throughout the industry globally, it will endow plant engineering with a valuable leverage for efficiency.

Mathematical engineering models, in industrial practice, have been sometimes seen as something causing delays, difficulties, and inconveniences rather than being efficient design tools. Statistical methodology however, is an exception. Statistical multivariate data analysis and factorial analysis have become popular in industry and widely accepted as a tool to optimize or at least improve processes. This is much helped by user-friendly software that are easy to learn and apply. Another reason is, of course, that it works and leads to reduction of costs. These methods can definitely be applied in bioreactor design and operation in a multitude of ways.

Another trend close to this is the perhaps unexpected support to design from the regulatory bodies by enforcing the pharmaceutical industry to apply the principles of quality-by-design (QbD) and process analytical technology (PAT) [41, 44]. As the terms imply, quality should be associated with the design and, as repeatedly declared, be built into the design of the process. It is done by clearly defining the interdependency of critical parameters with the help of statistical measures in order to determine the parameter space where quality is maintained. Especially statistical factorial methodology, the so-called design of experiments (DoE), is an efficient tool for achieving QbD. The PAT is the enabling tool for QbD. Besides techniques such as DoE, other analytical methods and means provide relevant data for the critical parameter in the process and product. Moreover, once the

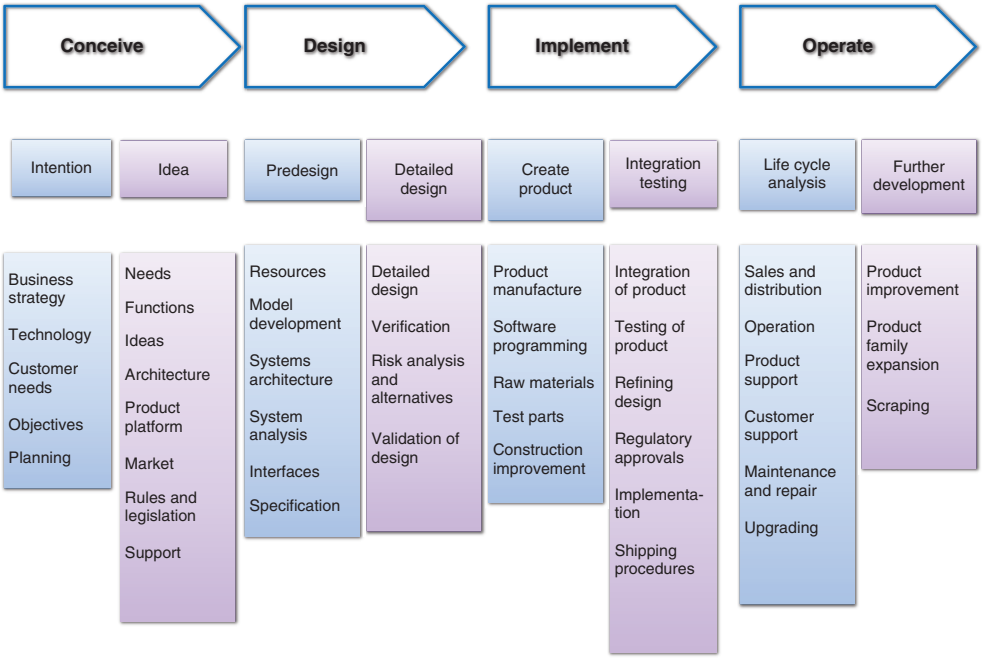

Figure 1.4 The CDIO concept: the process of developing a new product or production system is considered as a consecutive activity spanning from conceiving the product and production concept, designing the product or production system, implementing it into full-scale production, and finally operating it continuously for regular production. It is advocated that The CDIO is applicable to all industrial development work and should, therefore, be the framework for all engineering activity – from training and education till operating a process (from [18]).

design and operational space are set, the product should be controlled dynamically to stay within it. This requires both adequate monitoring methods and reliable control approaches. For this, general methodologies are available and applied in industry. QbD has become an integral part of regulatory work and used by the development teams in the pharmaceutical industry. Although differently termed in other biotechnology applications, such as in food, biochemical, and enzyme and bioenergy production, the same quality aspects and design and development methods can be applied and are probably applied to a large extent. The same can be expected for a majority of manufacturing industries.

As pointed out in Section 1.1, the chapters of this book cover the majority of these recent trends. Evidently, although these new trends have already to a large extent spread over the entire industry, they do to a high degree apply to the challenges of bioreactor design and operations. This is, in particular, true for conceptual design methodologies. Viewpoints on bioprocess unit design, bioreactors in particular, with a conceptual mind-set have gradually unfolded in recent years.

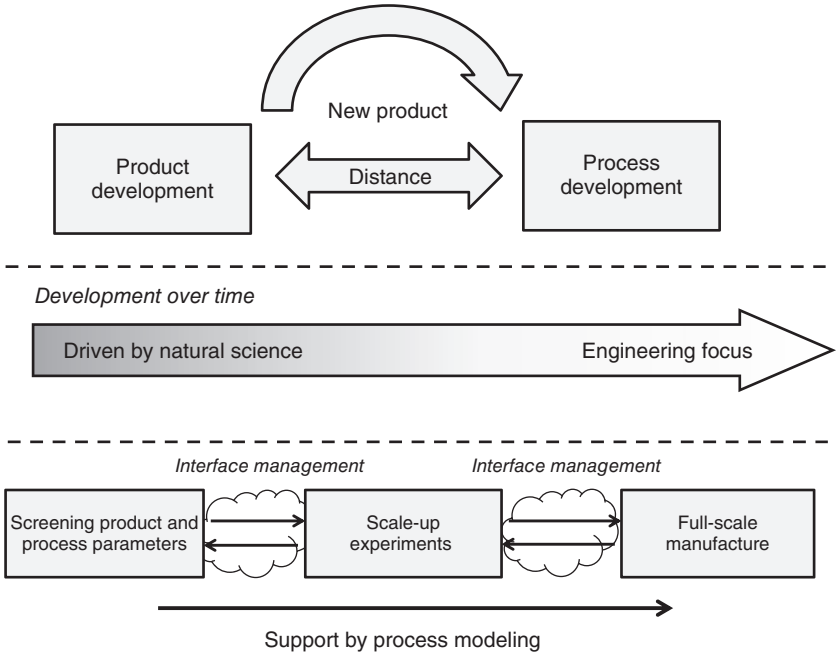

Figure 1.5 Framework for the development of a new product and its production depicted from three critical hurdles: the transfer of the product concept into a bioprocess, the transcendence of development from natural science to engineering, and the interaction between screening, scale-up, and full-scale manufacture. In all, the bioreactor has a key role (from [20]).

This has been facilitated and probably inspired by the access of other technical means and tools.

However, it needs to be stated that a multitude of challenges encompass bioproduct and process development are more demanding and diverse than in other areas of industry, although bioproducts also share a number of similarites with other industrial products. This has been illuminated by Neubauer *et al*. [20] by combining basic biochemical and bioprocess engineering with management technology perspective. Basically, the biotechnology industry faces three dominating challenges: transferring the complex biological understanding of a production organism and its target product into the engineering domain, addressing key engineering objectives that are already in early development phase, and speeding up the path from lab- and pilot-scale to full-scale manufacture (Figure 1.5).

New tools are available for meeting these challenges that may have substantial impact on the product and process development. These are micro-multi-bioreactor systems for early screening experiments, statistical methodologies for optimization of production methods, scaled-down [38, 40, 43] process unit systems and new sensors, and other bioanalytical instruments. These tools need to be placed on a convenient development platform where they are used

synergistically. Bioreactors, their design, and operation are key components in the approach, as high titers of target products are established thereby. However, the recovery of the target product should also be integrated into the platform approach to fully exploit the potential of the tools.

Undoubtedly, the majority of the trends discussed here are not restricted to biotechnology and bioreactors, which is of course a big advantage. Methodologies that are founded in industry in general are advancing more efficiently and faster. Biotechnology is small in comparison to other industrial activities and may benefit substantially from other adjacent technology areas.

1.5 The Systems Biology Approach

A most resourceful tool for the design and operation of bioprocesses, in particular for the bioreactor stage of a bioprocess, is systems biology, sometimes also termed systems biotechnology to highlight its utility for technical biological applications and distinguish it from biomedical or general applications in science. Probably no other current trends in modern biotechnology has the potential to impact bioprocessing as much, due to the process economic implications it could pave way for. The "omics" tools of systems biology have the power to substantially influence the designing of biological production systems, because they have, or at least have the potential, to measure minute events, rates, and metabolic flows within the cells that transcend other analytical means in consumed time and data volumes, and by that, reach parameters that can facilitate to effect better design and operation decisions of bioreactors. This concerns especially the biological functions that have a direct potential impact on the design of the bioreactor involving pathway engineering, transporter engineering, removal of negative regulation, and engineering of the regulatory network of the cell [50].

Objectives of systems biology typically aim at process intensification. If successful, it implies more demanding requirements of the transport of heat and energy, gases, and nutrients on the bioreactor system. Thus, efficiency of agitation and transfer becomes pronounced and calls for another round of optimization of engineering parameters. *In silico* simulations can be used to theoretically further predict, or verify, expected effects. The systems biology tools, or "omics" tools, are key in this, including genomic and transcriptomic arrays, proteomics tools for protein structure, metabolomics tools for pathway analysis supported by high-throughput instrumentation. This comes very close to the need of understanding of biological processes in the cell as is fundamental in medicine and for clinical purposes.

Park *et al.* [21] describe the systematic approach of using systems biology as a three-round procedure. Figure 1.6 illustrates the basic ideas behind the overall procedure of strain improvement by systems metabolic engineering. The factors to be considered are shown. In short, strain selection is followed by (i) the first round of metabolic engineering, which allows the development of a base strain.

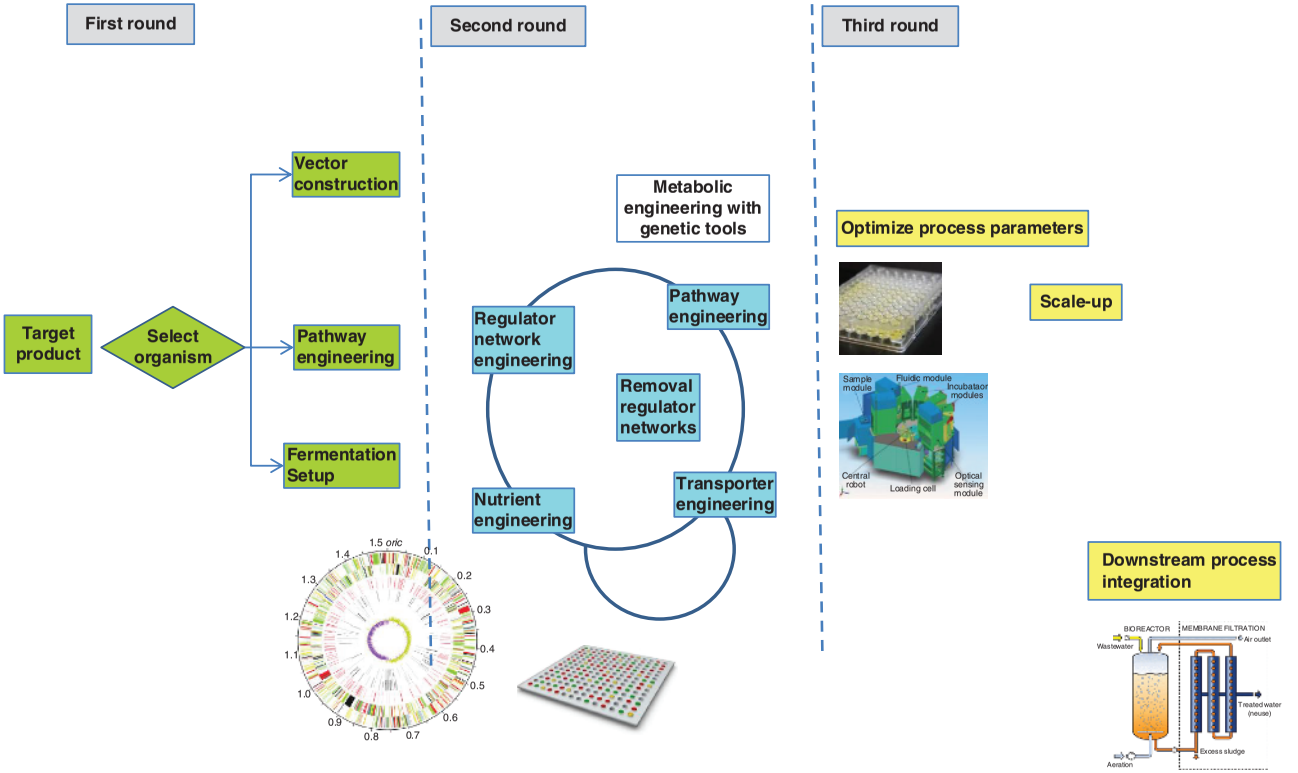

Figure 1.6 A systems biology approach for bioreactor process development based on a three-round procedures.

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].

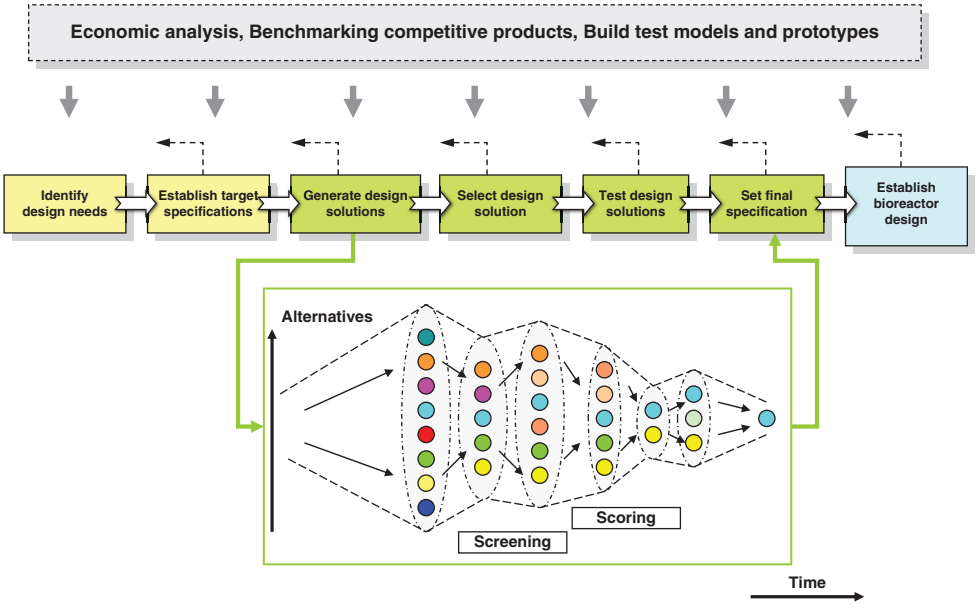

Figure 1.7 Conceptual design principle according to Ulrich and Eppinger sequential design concept where product alternatives are screened versus customer needs.

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

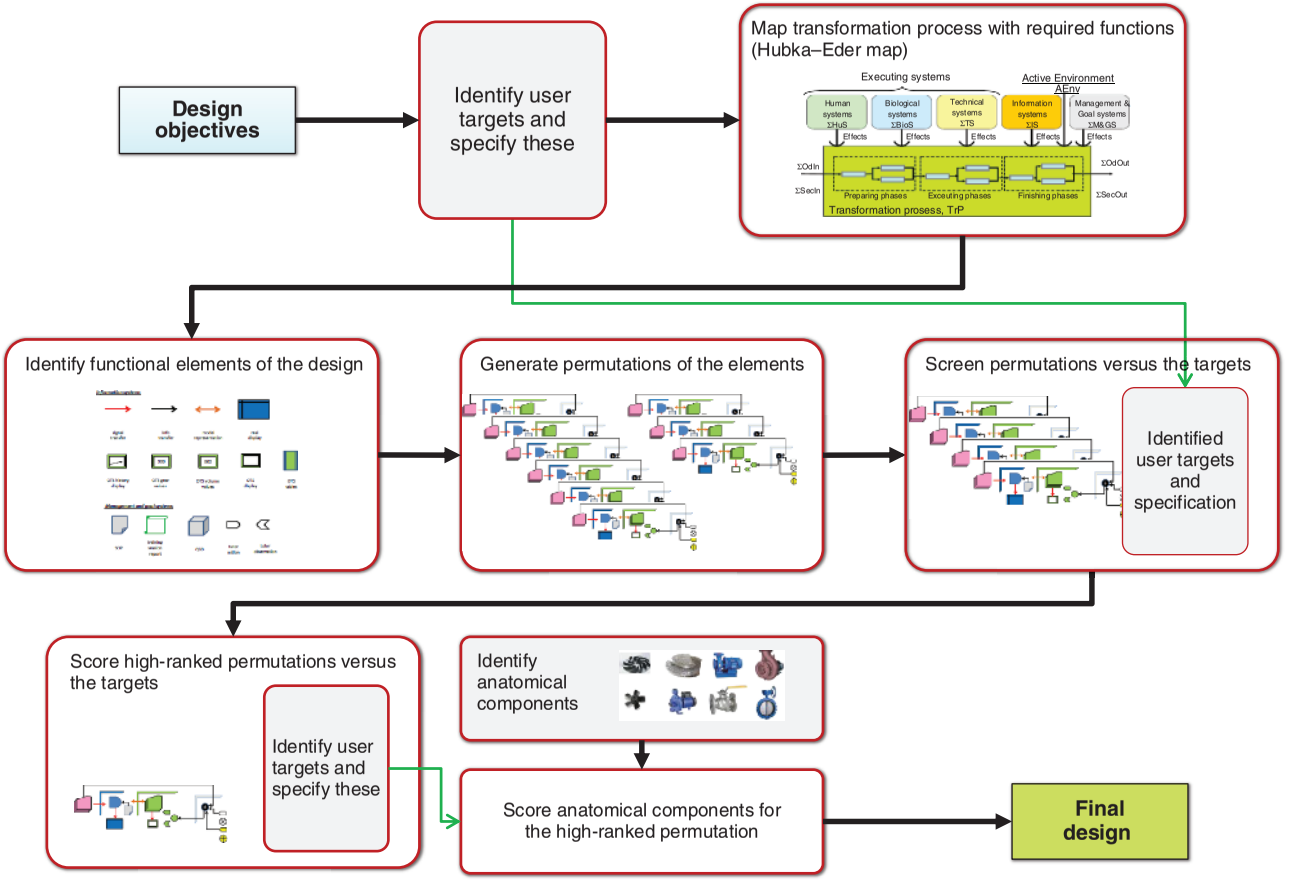

Figure 1.8 The biomechatronic methodology according to Mandenius and Björkman where conceptual methods and tools are combined in a complementary methodology.

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.

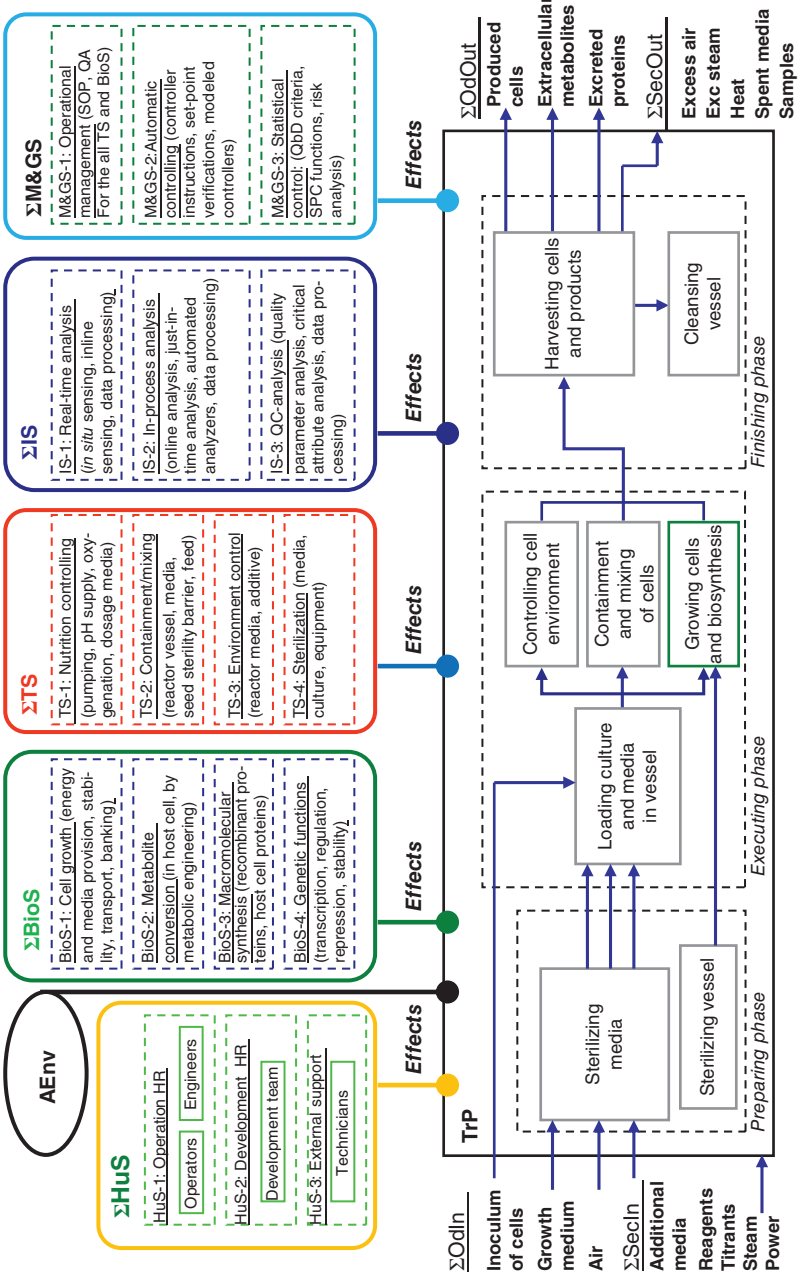

Figure 1.9 Hubka–Eder map of a bioreactor for the microbial production of a recombinant protein. Overall Hubka–Eder map showing the transformation process and the systems and subsystems involved for performing the transformation.

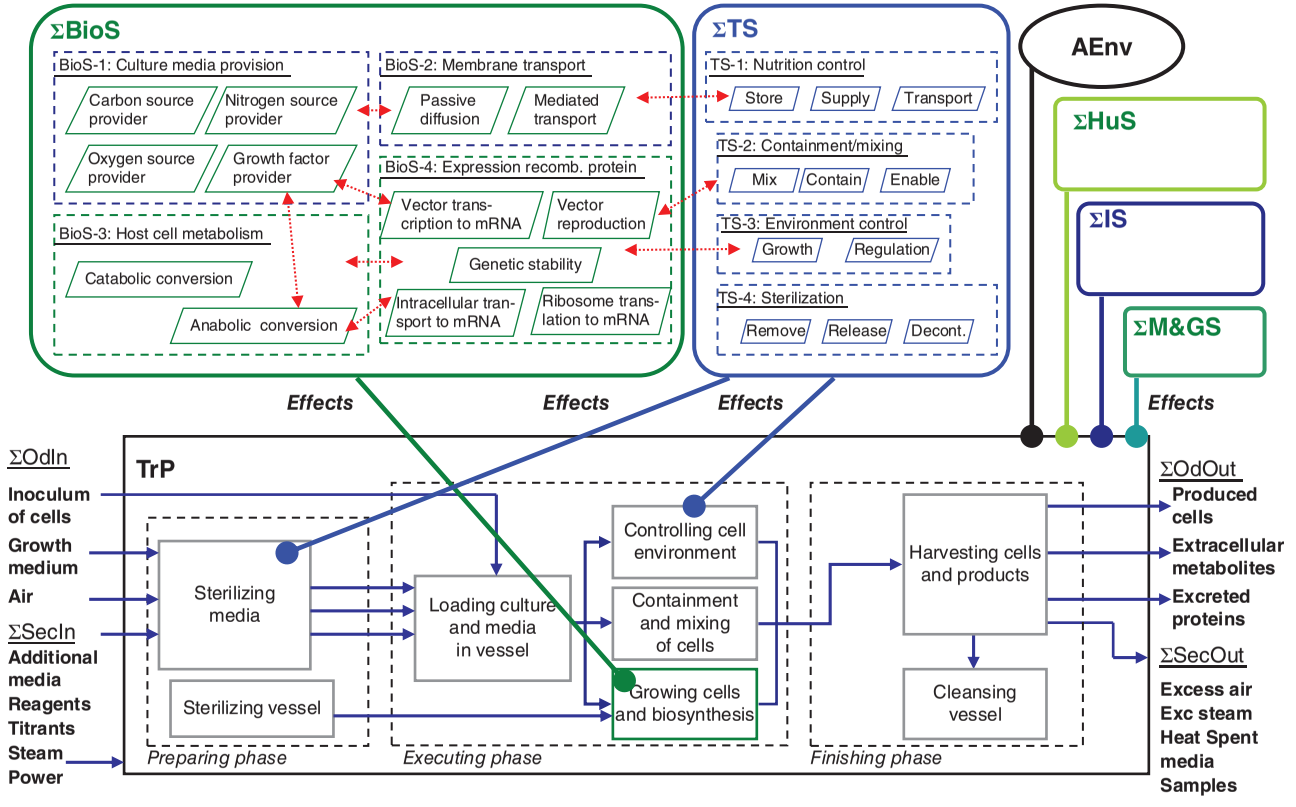

Figure 1.10 A zoom-in of the Hubka–Eder map in Figure 1.9 showing the ∑BioS and ∑TS systems and the interaction of subsystems.

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

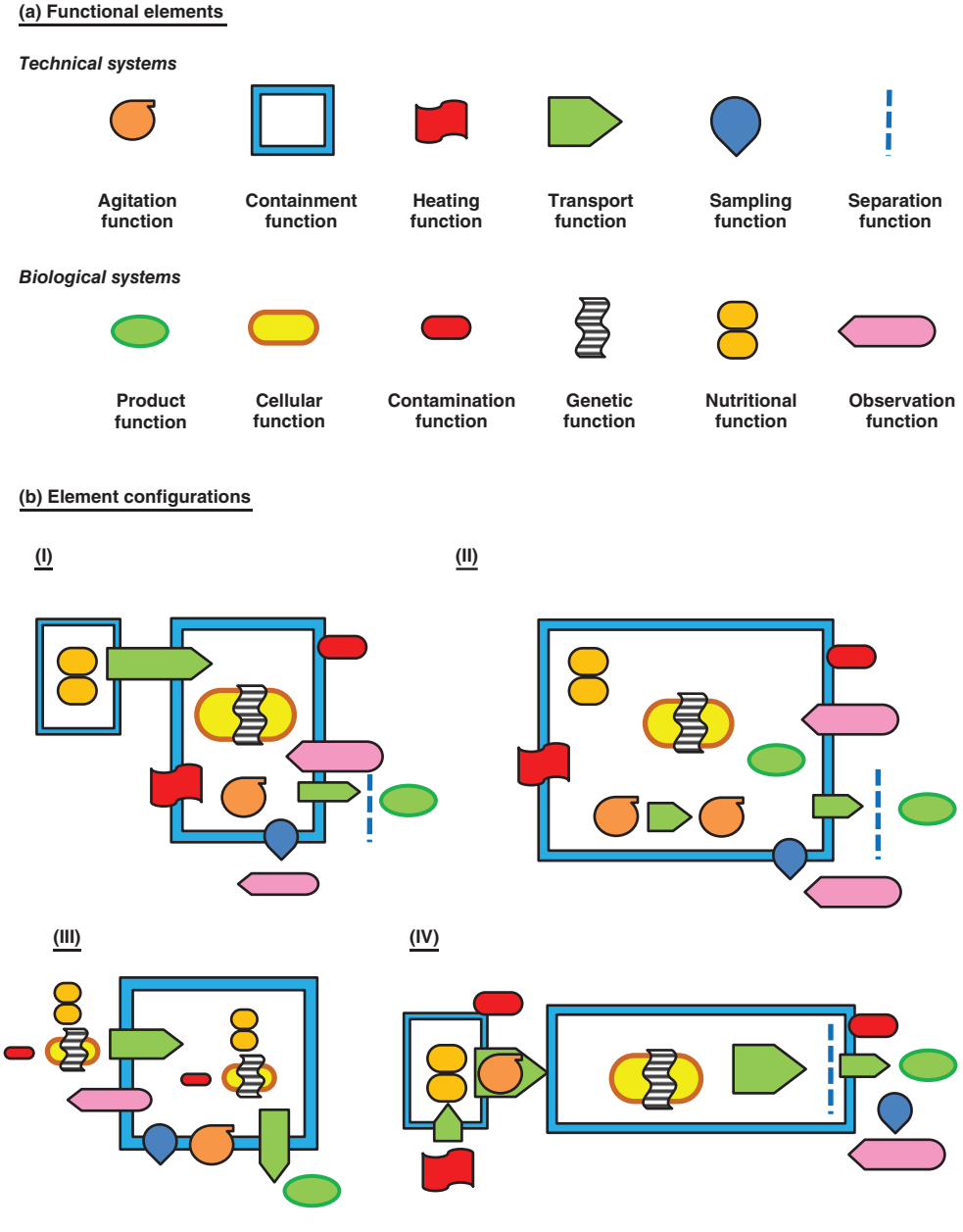

Figure 1.11 (a) Functional elements derived from the systems in the Hubka–Eder map. In a real design, the number of elements may exceed 100. (b) The elements are combined in order to envisage various configurations. The four examples shown can be configured in a variety of other permutations.

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

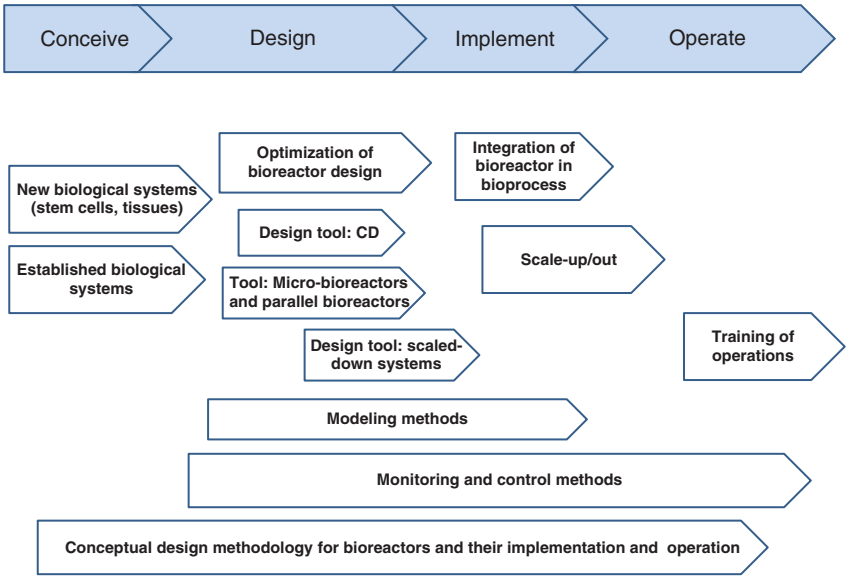

Figure 1.12 The CDIO concept as defined in Figure 1.4, here adapted to bioreactor design and operation with several of the topics and challenges addressed in this book.

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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