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Platform-Oriented Biopharmaceutical Process Design Using Novel Combinatory Methods

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Platform-Oriented Biopharmaceutical Process Design Using Novel Combinatory Methods

Dissertation

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Abstract

Biopharmaceutical products emerged as the principal driver for innovation in the pharmaceutical industry. Although of high economic and social importance, the development of biopharmaceutical manufacturing processes is still driven by time-intensive empirical approaches, delaying the time-to-market of drugs and jeopardizing the economic competitiveness of manufacturing processes.

A promising approach to speed up process development and to enable robust manufacturing is to build-up process knowledge to form well-characterized biopharmaceutical manufacturing platforms. This enables the efficient development of production processes for a broad spectrum of products while simultaneously drug product quality and economic manufacturing risks are reduced.

Within this thesis, the development of a novel and highly versatile *E. coli* recombinant protein production platform is presented. The built-up of platform process knowledge is achieved using novel combinatory methods which combine cutting edge technologies such as first-principle soft-sensors, dynamic experimentation, mid infrared and dielectric spectroscopy as well as multivariate data analysis and kinetic modeling.

Major novelties include the presentation of highly automated methods for the extraction of strain specific parameters, information which is essential for science-based bioprocess design. Expression tuning on cellular level is demonstrated using solely process technological means, resulting in a high degree of processing flexibility for the intended pharmaceutical manufacturing platform. Furthermore, for the first time, generic control methods based on soft-sensors are presented, which allow controlling multiple physiological bioprocess parameters simultaneously.

This thesis can be considered a case study demonstrating how combinatory methods can be purposefully exploited for the fast development of an efficient bioprocessing platform. The methodological focus of this thesis allows leveraging the developed combinatory methods and the platform bioprocess to other biotechnological manufacturing tasks and will enable the development of more competitive and predictable bioprocesses.

Introduction

Biopharmaceutical drugs are both of economic and societal importance, with global sales exceeding US $100 billion in 2010 [1]. By 2014, 26% of all genuinely new drugs approved in the United States were reported to be biopharmaceuticals [2]. Today, biopharmaceuticals are considered key drugs to fight diseases such as Alzheimer's disease [3] as well as cancer [4].

Biopharmaceuticals are defined as "A protein or nucleic acid based pharmaceutical substance used for therapeutic or in vivo diagnostic purposes, which is produced by means other than direct extraction from a native (non-engineered) biological source" [5]. Hence, the term "biopharmaceutical" refers to products such as therapeutic recombinant proteins (monoclonal antibodies, growth factors) as well as nucleic acids based pharmaceuticals (DNA and RNA).

As for any drug, market approval and manufacturing of biopharmaceuticals is under strict regulatory oversight to guarantee consistent product quality, safety and efficacy. In this context "quality" refers to identity, strength, and purity of the pharmaceutical drug product [6]. Compared to small molecule drugs, biopharmaceuticals show a higher degree of complexity, in particular in respect to the aggregation state, the complex three dimensional structure and the mode glycans are attached to the product which is referred to as glycosylation [7]. Therefore, proof and assurance of drug product quality is highly challenging, also due to analytical difficulties to determine structural properties of biopharmaceuticals [7]. This is also reflected by the fact that even approved biopharmaceutical products can show a relatively large variation in structural parameters, for example glycosylation [8].

In the recent years, the regulatory authorities started initiatives to urge industry to apply science- and risk based methods to achieve a state of "quality by design", meaning that product quality is guaranteed by the manufacturing process itself and not only assured by final product testing [9]. In this context, the regulatory authorities urge manufacturers to develop "process understanding" using risk- and science-based methods rather than empirical methods [10]. Process understanding can be interpreted as to have scientific insight how manufacturing process parameters effect drug quality attributes and, following an extended definition, also how manufacturing process parameters effect process performance attributes. This is especially important for the biopharmaceutical sector due to high structural complexity of the products being produced and the complex manufacturing processes involving living organisms (cells). Lack of process understanding can result in unwanted variations in drug quality and process performance, especially during scale-up of processes and manufacturing process changeovers. We anticipate that process understanding is not exclusively of regulatory interest: A high degree of scientific process understanding is also anticipated to decrease the number of failed batches, reduce unwanted scale-effects and also increase bioprocesses economics [11]. Hence, process understanding is also a business interest for the pharmaceutical industry.

Pharmaceutical process development

Process understanding can be achieved through sound risk- and science based process development [10]. Bioprocess development refers to all tasks aiming at the investigation and identification of an optimal set of process parameters for maximum productivity while meeting the desired product quality specifications. Furthermore, process development aims at the definition of control strategies to ensure predictable product quality and productivity throughout the process lifecycle.

In short, bioprocess development for pharmaceutical products involve following phases: First, high performing strains need to be identified in parallelized strain screening experiments. Subsequently, lab-scale experiments are conducted aiming at the identification of optimal process parameters, processing modes and the definition of control strategies. Then, the process is transferred to pilot-scale aiming at the identification of scale-dependent effects. Finally, the process is transferred to manufacturing scale. Process development is time-, cost- and labor intensive. To reduce the overall costs of biotechnological products and make bioprocesses more competitive, efficient strategies are needed to reduce process development time.

To reduce costs of biotechnological products and for the benefit of competitive and high performing pharmaceutical bioprocesses, bioprocess development should be fast and consume as few resources as possible. Current trends to accelerate bioprocess development are massive parallelization and miniaturization to perform high throughput experiments [12], more efficient experimentation using dynamic methods [13] and intensive real-time analytics and automated data mining [14]. These two approaches can also be summarized as a) increasing process development efficiency through doing more experiments (high throughput approach) and b) increasing the extractable information from experiments performed (dynamics and data mining approach).

These and other approaches can be used to build up systematic knowledge to establish a manufacturing platform. In turn, a manufacturing platform forms the technological basis for the production of a broad spectrum of products. In the biopharmaceutical manufacturing context, we define a bioproduction platform as the combination of technologies (platform technologies) and domain knowledge (platform knowledge). On this basis, a broad spectrum of bioprocesses can be designed. Within this thesis, the development of an *E. coli* bioproduction platform aiming at the production of recombinant proteins is in focus.

Features of a bioproduction platform

In the previous section, we defined a bioproduction platform as a combination of technologies (platform technologies) and domain knowledge (platform knowledge) aiming at efficient process design. Here, we introduce anticipated key features of a recombinant bioproduction platform.

Knowledge on process design constraints

Constraints for bioprocess design are manifold. A key feature of a bioproduction platform is to provide knowledge on these constraints. This in turn avoids design pitfalls from an early stage on. In this context, we differentiate three types of bioprocess design constraints.

Technical constraints: Bioprocess design is constrained by technical limitations of the bioreactor, such as maximum oxygen transfer rates or maximum heat transfer rates. We refer to the combination of process parameters that comply with technical constraints as the "technically feasible space".

Physiological constraints: Next to technical constraints, bioprocess design is constrained by physiological limitations of the microbial cell factory, for example maximum specific growth rates as a function of cultivation temperature or mixed feed metabolic capabilities. We refer to the combination of process parameters that comply with physiological constraints as the "physiological feasible space".

Product related constraints: Of course, bioprocess design needs to consider constraints concerning the product being produced, for example degradation of products at certain process conditions. These product dependent constraints can be derived using risk-assessment methodologies such as Ishikawa diagrams or failure mode and effects analysis. We refer to the combination of process parameters that comply with physiological constraints as the "product rational feasible space".

The combination of technical-, physiological- as well as product feasible space forms the process optimization space, which is illustrated in Figure 1. This is the starting point for empirical- or model driven lab-scale investigations to identify the relationships between process parameters and quality- as well as process performance attributes. The optimization space can be explored using a combination of parallel processing, design of experiments as well as mechanistic modelling.

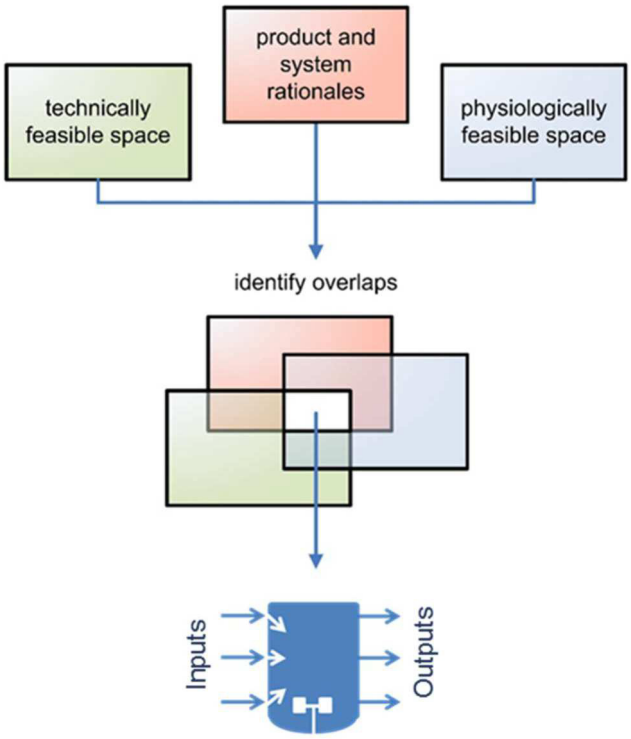

Figure 1: Bioprocess design is constrained by technical-, physiological and product constraints. Within the intersection of the technical-, physiological and product feasible space bioprocesses can be designed.

Versatility of the expression system

Production strategies for recombinant proteins are manifold: Recombinant proteins can be produced as highly pure protein aggregates known as inclusion bodies, requiring high level expression of the recombinant protein [15]. Other strategies involve targeting the periplasmatic space to different compartment of the cell factory, enabling for example the formation of disulfide bridges. This requires moderate expression levels for optimal performance [16]. Hence, in order to cover multiple expression strategies, a recombinant bioproduction platform needs to be able to be adjustable in respect to the expression level, which is also referred to as “expression tuning”. For maximum flexibility, a process-technological cog-wheel is necessary to tune the recombinant protein expression on cellular level to predefined objective without the need for genetic modifications. This concept is illustrated in Figure 2.

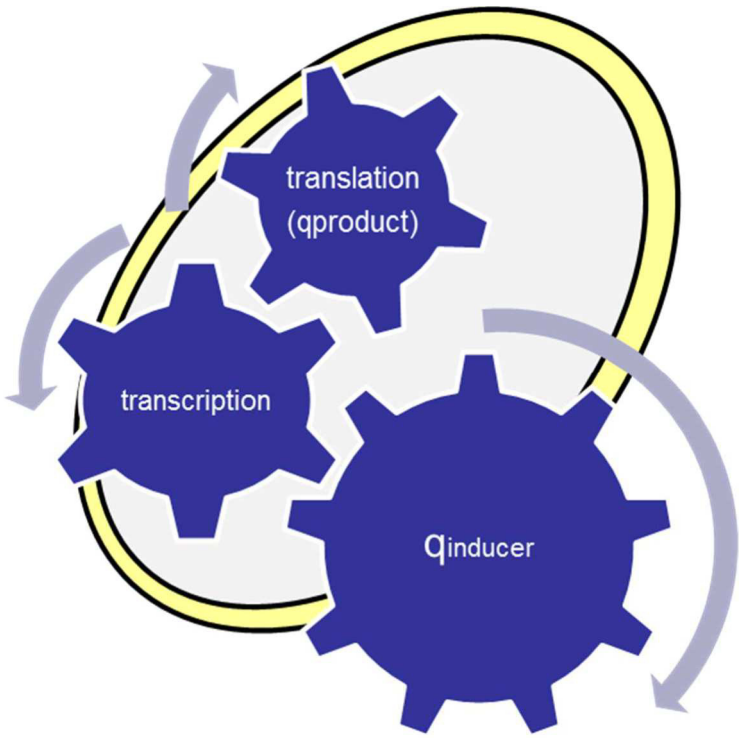

Figure 2: Tuning of recombinant protein expression on cellular level. A process technological modulator (e.g. the uptake rate of inducer $q_{inducer}$) drives transcription and therefore also translation of the desired product (qproduct). This enables a broad applicability of the expression system.

Platform technologies for process simplification

Integrated bioprocesses comprise multiple unit operations such as seed train, fermentation and multiple downstream unit operations. Each unit operation adds costs and complexity to the integrated bioprocess. Hence, technologies are needed that allow to simplify integrated bioprocesses, for example technologies enabling recombinant protein release to the extracellular space instead of using high pressure homogenization [17]. However, the introduction of a novel technology to an existing bioproduction platform adds novel constraints in respect to process design. For a sound integration of novel technologies in existing platforms, a sound investigation of technology-related constraints is necessary. This in turn allows to predictively assess benefits and drawbacks of the respective technologies.

Platform control strategies

For bioprocesses, environment process parameters such as temperature, pH and dissolved oxygen are monitored and controlled on a routine basis. For the benefit of more robust bioprocesses, a bioprocess platform can provide advanced platform specific control strategies. In this context, a control strategy should be generically applicable for all processes designed using the bioprocess platform.

Combinatory methods for the generation of platform knowledge

In the previous sections, characteristics and features of a recombinant bioproduction platform were described. As outlined, a recombinant bioproduction platform requires extensive system knowledge to enable efficient science-based process design. The acquisition of the required knowledge using traditional microbial methods (shake flask experimentation, chemostat experiments) is highly time- and cost intensive, reducing the overall efficiency of the production platform. Alternatively, we propose the combined use of cutting edge process development tools such as process simulation, process analytical technology, dynamic experimentation and mathematical-model based process control. The purposeful combination of these tools form what we refer to as “combinatory methods”, which allow the efficient and highly automated extraction of platform knowledge. A high degree of automation allows the extraction of platform knowledge without manual user interaction, which is also prerequisite for using combinatory methods for high-throughput strategies.

Using combinatory methods, platform knowledge is more efficiently generated and overall process development time is reduced. Figure 3 illustrates the proposed workflow for the extraction of platform knowledge using combinatory methods on the example of the extraction of a physiological constraint (temperature dependence of the specific growth rate).

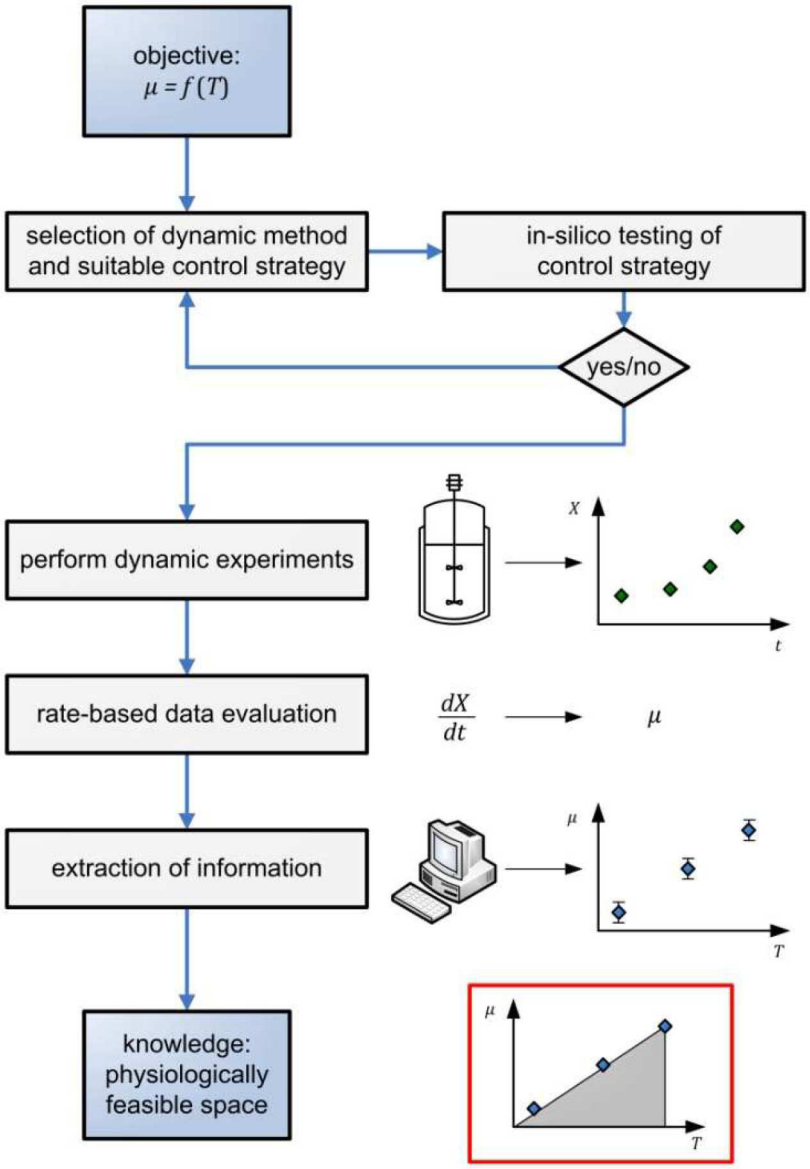

Figure 3: Based on a defined objective, e.g. the investigation of the temperature dependence of the specific growth rate, a suitable dynamic experimental strategy is selected. The respective experimental strategy is tested for applicability using computer simulation. Next, experiments are conducted and data is acquired using in-line or on-line analytical tools. The desired information is subsequently extracted using automated data processing tools. The extracted biological relationship (here: temperature dependence of the specific growth rate) describes a physiological constraint for the investigated system. This knowledge (physiological feasible space) is necessary for bioprocess design.

Platform-oriented bioprocess design using novel combinatory methods

In this thesis, the development of a novel and highly efficient bioproduction platform for the design of recombinant bioprocesses using novel combinatory methods is demonstrated. Figure 4 depicts the classification of contributions that are part of this thesis. Table 1 to Table 3 summarize the contributions that are part of this thesis.

First, a set of review contributions and a book chapter summarize the current state of the art for the design of recombinant processes and key platform technologies (online monitoring and tunable recombinant protein expression). Then, novel fundamental technologies for bioprocess analysis are presented: We describe the extraction of physiological knowledge using novel multivariate data analysis approaches based on physiological information [18], novel methods for the real-time monitoring of bioprocesses using mathematical models [19] as well as a fundamental study dealing with the propagation of error on data to the extracted biological information [20]. Based on these fundamental tools, combinatory methods are presented that efficiently allow to extract platform process knowledge. Physiological knowledge such as the onset of acetate production [21], physiological changes during process phase transitions [22], the temperature dependence of the specific growth rate [23] and the ability of co-utilization of multiple substrates were investigated using the combination of process simulation, process analytical technology, dynamic experimentation and mathematical-model based process control. Fundamental novel insight in respect to tuning on cellular level for the L-arabinose utilizing pBAD mixed feed expression system is reported [24], demonstrating for the first time expression tuning on cellular level in *E. coli* using a mixed feed approach. Studies were performed resulting in the development of scientific rationales for bioprocess feeding design [25]. Furthermore, process simplification is demonstrated by integrating a novel platform technology (protein E mediated cell lysis) to the developed and characterized bioproduction platform [26].

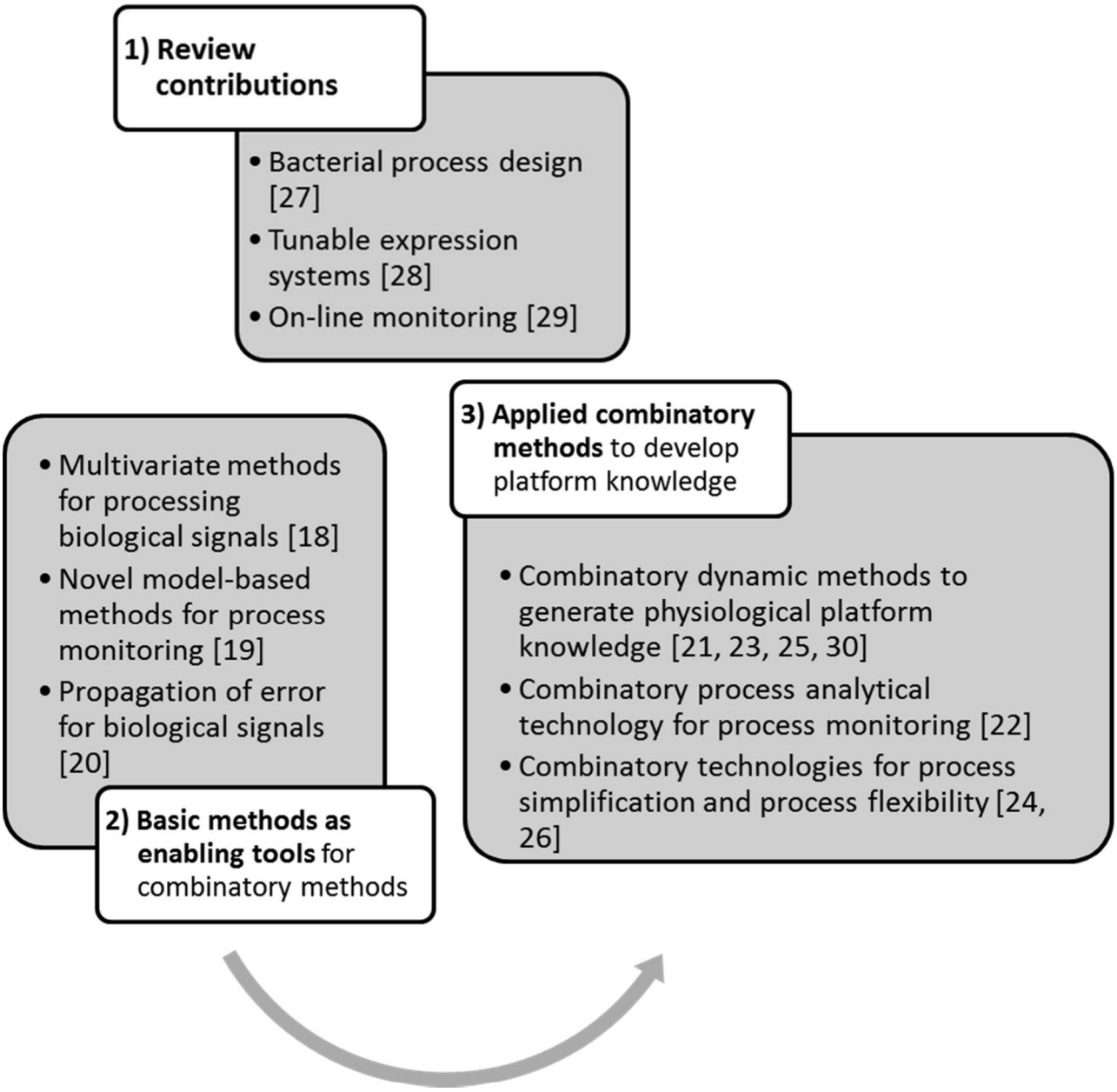

Figure 4: Classification of contributions covered within this thesis. Review contributions summarize the state of the art. Fundamental methodological research resulted in the development of "basic methods" (e.g. novel algorithms). These methods are enabling tools that for combinatory methods, which were applied to develop platform knowledge for the recombinant bioproduction platform.

Table 1: Review contributions summarizing the state of the art of the scientific disciplines in focus

1) Review contributions	Topics discussed and reviewed
Bacterial suspension cultures [27]	State of the art of bacterial bioprocess design
Tunable recombinant protein expression in *E. coli* [28]	State of the art of tunable recombinant protein expression in bacteria
Ex-situ on-line monitoring and data processing [29]	State of the art of ex-situ on-line monitoring for process design-, analysis- and control purposes

Table 2: Basic methods as enabling tools for combinatory methods

2) Basic methods as enabling tools for combinatory methods	Basic methodology developed
Information processing [18]	Rate-based multivariate analysis of fermentation process data sets
Real-time estimation of biomass [19]	Strategies and limitations for the sound estimation of biomass and the specific growth rate
Information from Data [20]	Sampling intervals to achieve uniform signal to noise ratios for rate based information

Table 3: Applied combinatory methods to acquire process knowledge

3) Applied combinatory methods	Developed Process knowledge
Volumetric mass density [22]	Physiological adaptation from fed-batch to induction phase were investigated using a novel combinatory method based on dielectric spectroscopy and soft-sensors
Efficient feeding profile optimization [25]	Memory effects from fed-batch to induction phase were investigated using a multivariate study
Soft sensor control [21]	Overflow characteristics (acetate production) were quantified using soft-sensor assisted dynamic methods
Soft sensor for mixed feed [30]	pBAD mixed feed catabolite repression characteristics were quantified using soft-sensor assisted dynamic methods combined with in-line spectroscopy
Induced metabolic capacity [23]	Maximum specific growth rates as a function of temperature were quantified using soft-sensor assisted dynamic methods combined with in-line spectroscopy
Tunable expression pBAD [24]	Expression tuning on cellular level was quantified and proven for the pBAD mixed feed system via a multivariate study
Protein E I.B. processing [26]	Physiological constraints for the use of the protein E technology in combination with the pBAD platform were quantified via a multivariate study

Methods used within this contribution

Design of Experiments, in-line spectroscopy, dynamic methods, control strategies, information mining and mechanistic modelling form the methodological basis of this thesis. Furthermore, so-called soft-sensors were frequently applied, which allow to estimate non-measured process variables using the available process data in combination with a mathematical model.

These methods were purposefully combined to novel combinatory methods. Table 4 provides an overview of the methods used within the contributions. This enabled i) efficient and automated extraction of platform process knowledge and ii) the development of novel platform technologies for process design flexibility and robust manufacturing.

Table 4: Methods used within this contribution

Methodological Contributions / Methods	Design of Experiments	Soft sensors	In-line Spectroscopy	Dynamic methods	Control Strategies	Information Mining	Mechanistic Modeling
Information processing [18]						■	
Real-time estimation of biomass [19]	■	■					
Information from Data [20]						■	■
Volumetric mass density [22]	■	■	■				
Efficient feeding profile optimization [25]	■					■	
Soft-sensor control [21]		■		■			
Soft-sensor for mixed feed [30]		■	■	■			
Induced metabolic capacity [23]		■	■	■	■	■	■
Tunable expression pBAD [24]	■					■	
Protein E I.B. processing [26]	■	■	■	■	■	■	■

Author contributions

Table 5, Table 6, and Table 7: Author contributions

1) Review contributions	Contributions of Patrick Sagmeister (PS)
Bacterial suspension cultures [27]	PS conducted the literature research and drafted the major sections on bacterial process design, quality by design and single use bioreactors
Tunable recombinant protein expression in *E. coli* [28]	PS drafted the manuscript and contributed to literature research
Ex-situ on-line monitoring and data processing [29]	PS drafted the sections on data and information processing: Data collection & alignment, Data preconditioning & rate calculation, Data reconciliation, Information processing, Rate-based information processing, soft-sensors

2) Fundamental methods as enabling tools for combinatory methods	Contributions of Patrick Sagmeister (PS)
Information processing [18]	PS performed the lab experiments, data analysis and drafted the manuscript
Real-time estimation of biomass [19]	PS contributed to the data analysis and experimentation and helped to draft the manuscript
Information from Data [20]	PS contributed to the data analysis and experimentation and helped to draft the manuscript

3) Applied combinatory methods	Contributions of Patrick Sagmeister (PS)
Volumetric mass density [22]	PS conducted data analysis and drafted the manuscript
Efficient feeding profile optimization [25]	PS conducted parts of the experiments, contributed to data analysis and helped to draft the manuscript
Soft sensor control [21]	PS designed the experiments, performed the experiments, conducted data analysis and drafted the manuscript
Soft sensor for mixed feed [30]	PS designed the experiments, conceived the control strategy, performed the lab experiments, data analysis and drafted the manuscript
Induced metabolic capacity [23]	PS designed the experiments, conceived the control strategy, performed the experiments, conducted the data analysis and drafted the manuscript
Tunable Expression pBAD [24]	PS designed the experiments, performed parts of the experiments, conducted the data analysis and drafted the manuscript
Protein E I.B. processing [26]	PS designed the experiments, performed parts of the lab experiments, conducted data analysis and drafted the manuscript

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Summary and Outlook

Summary

pBAD mixed feed bioproduction platform

Within this thesis, a novel and highly powerful bioproduction platform for the production of recombinant proteins was developed. The developed bioproduction platform shows unique features such as the ability to tune recombinant protein expression to the demand of the host cell, thoroughly characterized strain physiology for science-based process design and tailored technologies for process simplification (protein E mediated cell lysis) and process monitoring and control (soft-sensors and soft-sensor control strategies). For all technologies, both benefits and limitations are well characterized. The power of the bioproduction platform was demonstrated for the production of one model protein (green fluorescent protein) and one highly relevant recombinant human growth factor (recombinant human bone morphogenetic protein).

Fundamental insights in the field of fermentation data processing

For the development of the novel bioproduction platform, fundamental research in the field of fermentation data processing was carried out: Novel multivariate data analysis methods based on physiological information [1] and novel soft-sensor based process monitoring methods [2] were suggested. Furthermore, for the first time general investigation of error propagation from data to rate based physiological information were made [3].

Combinatory methods for process design, analysis and control

Cutting edge process analytical, automated information extraction and soft-sensor assisted control methodologies were developed and purposefully combined (combinatory methods). The power of the developed novel combinatory methods was demonstrated for a) the efficient development of the necessary platform knowledge for process design, b) the characterization and integration of novel technologies for process simplification and design flexibility and c) the development of novel control strategies for robust manufacturing. The use of combinatory methods sets novel directions for the acceleration of bioprocess development.

Outlook

Transferability of the developed methods

Today's economy and wealth is heavily dependent on non-renewable resources, resulting in increasing ecological, economic and political challenges. In an attempt to progress economy to a state of sustainability, there is a drive to substitute non-renewable products and energy with bio-based alternatives [4]. In turn, this should lead to a "bioeconomy", which will enable the efficient conversion of waste streams and renewable resources to high value products and energy, reducing society's dependency on fossil resources. Next to the substitution of existing products through sustainable alternatives, bioeconomy is expected to fuel the development of novel innovative products.

Industrial and agricultural bioprocesses will play a key role on the way to the development of a bioeconomy. As for biopharmaceutical processes, empirical approaches are still prevailing for bioprocess development and in manufacturing [5]. Changing this current state of empirical-driven bioprocess development and manufacturing to a state of predictability and knowledge driven processing is declared goal both in the sectors of agricultural and industrial biotechnology. This will increase the competitiveness of agricultural and industrial bioprocesses and reduce process related product variation.

Within this thesis, the power of the developed combinatory methods were applied for the development of a biopharmaceutical manufacturing platform. However, it is strongly anticipated that the developed combinatory methods for process design, analysis and control can be leveraged to bio refinery- as well as industrial biotechnology processes which use cells as production units. This is possible due to the strict use of mechanistic approaches such as mass balancing and kinetic modelling which are used in the presented methods. For example, first-principle soft-sensors based on elemental balancing are also applicable for industrial biotechnology processes, whereby here next to the control of physiological parameters also the estimation of product can be addressed.

In analogy as demonstrated for biopharmaceutical processes, a significant reduction of process development times as well as a more robust bioprocessing is expected. Thereby, the developed methods will allow to increase the overall process efficiency and therefore also contribute to the development of a competitive bioeconomy.

Industrial implementation of methods

Within this thesis, it was exemplified how mechanistic combinatory methods can be used to systematically generate platform knowledge and thereby develop a novel bioproduction platform which is capable of accelerating bioprocess development. Although the benefits for industry were clearly demonstrated (faster process development, more robust bioprocesses), industrial implementation of the presented tools will be challenging due to high methodological complexity and the sophisticated interplay of a broad spectrum of tools (chemometrics, mechanistic modelling, sophisticated process analytical technology, control methods). Therefore, in order to unfold a societal and economic benefit, clear strategies for the industrial implementation are necessary. Potential industrial implementation scenarios are briefly discussed in this section and summarized in Figure 5.

Combinatory dynamic methods can be implemented in lab-bioreactor control software: Industrial users can then call automated workflows for defined bioprocess development tasks, for example investigation of induced state metabolic capabilities [6] or the onset of catabolite repression [7].

Generic soft-sensor assisted control strategies [8] can be implemented in lab-bioreactor control software for process development purposes. To unfold the full potential for these control tools, transfer to pilot- and manufacturing scale will be necessary. This in turn demands implementation on a SCADA (supervised control and data acquisition) or PLC (programmable logic controller) system.

The wealth of platform knowledge generated using the presented tools will result in further challenges in respect to data-, information- and knowledge management. Software tools (intelligence systems) will be necessary to efficiently extract relevant information from process data historians, for example using the information mining approach presented within this thesis [1]. Furthermore, tools for the management of mechanistic process models will be necessary which guide the industrial users in respect to model development, model maintenance and use of mechanistic models for simulation [3, 6] or also model-based process optimization purposes [9].

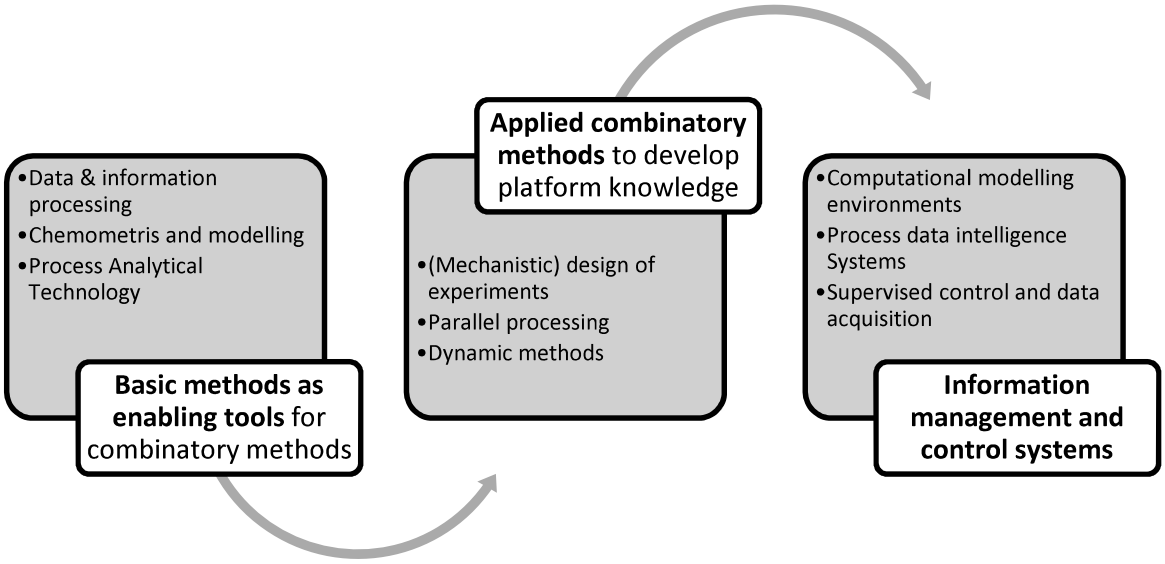

Figure 5: Within this thesis, basic methods as enabling tools for combinatory methods as well as applied combinatory methods were presented. In order for these methods to unfold their full benefits in industry, implementation in industrial information management and control systems is necessary.

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