Progress

Priorities for Addressing **O**pportunities and **G**aps of Industrial Biotechnology for an efficient use of funding **res**source**s**

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About PROGRESS

PROGRESS is a coordination and support action for the European Commission and aims to support and accelerate the deployment of IB in the EU industry by identifying high-value opportunities for IB and proposing actions to address them successfully. For that purpose, we will first provide a comprehensive and dependable information base (including modelling and simulation approaches) which allows for plausible estimations on the future of IB in the EU in the short and medium-term. Second, we will elaborate in collaboration with stakeholders a future scenario and a common vision for IB in Europe containing most promising value chains, related R&D&I needs and necessitated policies for IB in Europe. Based on these steps, we will provide strategic advice for research, industry and policy regarding potential issues/topics for collaboration, future policy programmes, the required technological infrastructure, capabilities, and economic structures. A main focus will be to identify opportunities for collaboration between EU member states and proposed actions to increase awareness and incentives for those collaborations. For more information see www.progress-bio.eu

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Content

1 Introduction

Biological systems, including living cells and components derived from these, have been used in productions processes for centuries especially for making food and beverages (i.e. for brewing and baking, or cheese making). However, while in these classical areas biotechnological application have evolved rather randomly lacking knowledge of the biologically active agents involved modern industrially biotechnology (IB) aims for a targeted application of biological functions in the production process. Thus, modern biotechnology is knowledge-driven and applies scientific principles from life sciences such as microbiology, molecular biology, and biochemistry as well as engineering. While the classical applications still make up the major part of the industrial biotechnology sector the extraordinary scientific developments in biotechnology are strong innovation drivers and have yielded a large spectrum of new and highly innovative applications for IB. In particular the rapidly growing capabilities in bioinformatics, DNA sequencing and DNA synthesis as well new tools for genetic manipulations are offering yet new technological opportunities which may allow for IB to enter new field of applications but also to enable the valorization of novel feedstocks to materials, fuels, and chemicals in order to decrease the need for fossil feedstock and avoiding competition with food and feed chains.

Any industrial production process is based on a feedstock from a given source that undergoes multiple conversion steps to yield an industrially relevant product. Within this production chain one or several conversion steps could be catalyzed by a biological agent (either an organism or isolated components therefore like enzymes), making it a biotechnological production step. Typically, biotechnological productions steps are combined with non-biotechnological conversions, including mechanical, chemical, or thermal. In a narrow sense industrial biotechnology will only include production processes where a central conversion steps is performed biotechnologically. However, there are numerous industrial processes that employ a biotechnological step either a part of the up-stream processing (USP) or the down-stream processing (DSP). Thus, biotechnology can – in theory play a relevant role in virtually any industrial field by adding new functions or replacing single steps in the production chain.

This report will describe the present state of the art, the scientific and technological trends in IB and the innovation potentials linked to these. Beside the basic principles and approaches which are fundamental to most IB production processes also novel emerging trends are presented that are presently receiving high attention within the scientific community and are expected to have great impact on IB innovation activities in the future.

2 Approach

The R&D topics presented in this chapter were identified based on a literature review. Moreover, interviews with stakeholders from various IB fields were conducted. The aim of these interviews is to confirm the findings from literature review, to identify topics that are of particular relevance for Europe and to capture also potential topics which might be underrepresented in scientific literature but of high importance for the development of the IB industry in Europe. This report represents a non-exhaustive overview on the existing and future technological trends in IB.

3 Biotechnological production platforms

Biotechnological production processes make use either of complete metabolic pathways taking place in living cells or only isolated parts thereof, i.e. in the case of enzyme catalyzed conversions (Aichinger et al. 2016). Generally, one can distinguish between production platforms that employ living cells (also referred to as organism- or cell-based systems) or those that are “cell-free”(also referred to as *in vitro* systems) applying only parts from cells (i.e. cellular extracts or isolated enzymes). While in the former case the complete cellular metabolism takes places it the latter case only a defined part of the cellular metabolism is used.

3.1 Cell-based production systems

Living organisms are capable for synthesizing a broad spectrum of compounds including proteins (enzymes and structural proteins), small molecules like amino acids and various metabolites, polymers, or secondary metabolites like hormones or antibiotics. As living organisms they provide not only the whole machinery for synthesis but they are also capable of self-regeneration (growth, replenishing of cofactors and reduction equivalents) and repair if provided with a suitable source of carbon and energy. While microorganisms undoubtedly play an important role in IB, various other production systems including algae and mammalian cells are used for commercial applications.

3.1.1 Microbial production systems

In industrial biotechnology, microbial production plays a prominent role. This goes as far as that industrial biotechnology is often understood as microbial fermentation. Despite the sheer enormous range of biological diversity found in nature only a rather small set of microorganisms has been exploited for the industrial production of chemicals and biofuels (Gustavsson, Lee 2016; Aichinger et al. 2016).

The production organisms used today have originally been isolated from nature due to their specific metabolic features and have been gradually optimized to fulfill the requirements of industrial production process. As a result of these intensive efforts, many of these organisms are now well studied model organisms for which large amounts of data exist as well as highly sophisticated tools for their manipulation and fermentation. Among the most important strains that are routinely used for industrial applications are bacteria, yeast, and filamentous fungi. A non-exhaustive overview is shown in Table 1.

Table 1: Overview of relevant microbial production strains and their applications. (Own compilation)

Strain	Characteristics	Relevant products
Bacteria		
Escherichia coli	"workhorse of biotechnology", most extensively studied organism	Amino acids, biofuels, chemical building blocks, polymers, pharmaceuticals
Corynebacterium glutamicum	Genetically engineered strains with broad substrate spectra, i.e. for use in biorefineries	Amino acids, vitamins, organic building blocks
Bacillus subtilis	Free of exo- and endotoxins, "GRAS" (generally recognized as safe", thus applicable for food and health applications Model organism for proteome studies Secretion of proteins into medium	Industrial enzymes
Yeast		
Saccheromyces cerevisiae	Traditional biotechnological yeast. "GRAS" (generally recognized as safe", thus applicable for food and health applications Aerobic and anaerobic growth, robust, large product range.	Food and beverages, biofuels, chemicals, materials

Pichia pastoris	Fission yeast, methylotroph (i.e. can grow on methanol), reaches high cell densities	Biopharmaceuticals, industrial enzymes
Filamentous fungi		
Aspergillus niger	Aerobic fermentation, high pH tolerance	Citric acid, gluconic acid, enzymes
Penecillium chrysogenum		Antibiotics, used for pulp mill treatment, enzyme production

Typically, these strains are also the starting point for **new production strains**. Even if new strains with promising characteristics are isolated from nature it is a common approach to transfer the relevant parts of the metabolic pathway into a well established industrial strain. Thanks to enormous developments in the field of genetic engineering and synthetic biology, the technical capabilities to introduce new metabolic functions into well characterized production strains have greatly improved in the past years (see also section 4). The vast majority of production strains that are used today have been derived from a relatively small set of model strains leading to innumerable variants, many of which are owned and protected by the company employing them or under the exclusive license of IB companies.

As an alternative approach to genetic modifications and optimization of model production organisms, natural samples or strain collections can be screened for completely new strains that have not been exploited industrially so far. This can be a relevant strategy, when genetic modifications are not desired. However, despite the great potential seen in natural diversity, the industrial exploitation of a “wild” strain typically requires intensive process development and optimization.

For certain fields of application also **safety aspects** play a central role. Even though industrial production typically takes place in a closed environment, with respect to health and environmental safety it is often required that the production strain does not produce any toxic by-products. This is particularly important for those organisms that are used in the food and feed production. The US Food and Drug Administration has attributed the “GRAS” (*generally recognized as safe*) designation to several microor-

ganisms or products that contain these, thereby declaring them as safe for human consumption1.

At present, there is an enormous potential to either isolate or to design new production strains. With the worldwide efforts to establish a biobased economy, there is a large demand for cheap and robust biotransformation systems. Therefore, there is a great potential to exploit the natural diversity of microorganisms, i.e. by identifying metabolic pathways that enable the utilization and valorization of alternative carbon sources (see section 5) such as waste products or green house gases like methane or carbon dioxide methanotrophs (Strong et al. 2015).

Anaerobic microorganisms, i.e. microorganisms that do not require atmospheric oxygen for the maintenance of their metabolism are used for much less industrial applications than aerobic microorganisms. At present anaerobic fermentation is mainly employed within environmental applications such as waste treatment. However, anaerobes exhibit several characteristics making them attractive also for a broad range of industrial applications including the production of chemicals and high value proteins (Hatti-Kaul, Mattiasson 2016; Mamo 2016). For one thing, oxygen intake is typically a limiting factor in aerobic fermentation processes. Moreover, a diverse spectrum of biological function is provided by anaerobes which have received little attention until now but present highly promising opportunities for IB.

3.1.1.1 Multiplex microbial consortia

Mixed microbial consortia in nature are capable of performing highly complex metabolic conversions yielding a plethora of naturally occurring compounds. Therefore, great potential is seen in the co-cultivation of micro-organisms (Bader et al. 2010; BIO-TIC 2015a). However, modern IB processes usually employ only a single, highly optimized production strain. Until now only naturally occurring co-cultures have been exploited industrially, for example in environmental application like waste water treatment, or food and biogas production. R&D in these application areas is typically driven by engineering disciplines whereas new biology-driven tools and approaches (e.g. sytems biology, metabolic engineering, synthetic biology) have received little attention in this field so far. Therefore, the underlying regulatory networks and mechanisms that control the interaction and communication between different species in multiplex microbial

1 http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/MicroorganismsMicrobialDerivedIngredients/default.htm. Retrieved September 8th, 2016.

consortia are still poorly understood. In order to broaden the application areas of consortium fermentation an interdisciplinary approach is needed which links the expertise from the “classical” applications of microbial consortia and the state-of-the-art technologies from bioinformatics, metabolic engineering and systems biology. For example as a prerequisite for rational engineering of microbial communities, systems biology approaches have to be developed further so that they can also be applied to complex consortia to order to identify and understand the interaction mechanism (Song et al. 2014; Zomorrodi, Segre 2016).

3.1.2 Microalgae

Microalgae have gained more and more attention as production organisms in the past years. As green plants, algae are photoautotrophs, meaning they can use sun light as energy and carbon dioxide as carbon source. Therefore, they do not compete with the food and feed chain. Microalgae can be used for the production of numerous high value products including fatty acids, proteins and pigments but also fine and platform chemicals like monomers for bioplastics and biofuels (Bellou et al. 2014; DECHEMA-Fachgruppe "Algenbiotechnologie" 2016; Lee et al. 2015; Milledge 2011). In addition, microalgae can be employed for environmental applications, like waste water treatment or carbon sequestration from exhaust gases (Sayre 2010; Bellou et al. 2014; Cheah et al. 2016; Delrue et al. 2016). Due to this versatile spectrum of potential products also biorefinery concepts based on microalgae have been proposed (Cheah et al. 2016; DECHEMA-Fachgruppe "Algenbiotechnologie" 2016).

However, compared to established production systems, algal biotechnology is still in its infancy. At present, the huge potentials that have been anticipated for microalgae have merely been exploited. Large scale investments including the establishment of biological databases, the development of tools for strain design and manipulations as well a bioprocess engineering will be needed to make algal production systems competitive for industrial applications (DECHEMA-Fachgruppe "Algenbiotechnologie" 2016; Valverde et al. 2016).

National and European funding agencies are currently supporting multiple national and multinational projects which aim at the establishment of integrated algal biorefineries. However, beside scientific and technological hurdles high economic constraints must be overcome (Godman et al. 2013). In the last decade research on algal production of biofuels has grown immensely. However, these efforts were largely policy-driven and it

has been questioned whether it makes economical sense to produce biofuels from algae rather than food and feed (Valverde et al. 2016).

3.1.3 Mammalian production

Mammalian cell cultures – e.i. immortalized cell lines which are typically of human or rodent origin – are mainly used for the production of high value proteins, including biopharmaceuticals like antibodies, hormones, or therapeutic enzymes (Bandaranayake, Almo 2014; Berlec, Strukelj 2013).

Among mammalien expression systems, Chinese Hamster Ovaries (CHO) cells are the most frequently used host for industrial production of recombinant proteins (Fischer et al. 2015). Mammalian cells are considered superior to microbial production systems with respect to protein folding and post-translational modifications. But despite great immense progress in the understanding of cellular regulation and the capabilities to genetically manipulate mammalian cells productivity of mammalian systems still lack behind in yield, typically reaching only less than 10 mg/mL (Lewis et al. 2016; Fischer et al. 2015). Despite these improvements, the cultivation of mammalian cells remains challenging and expensive. Industrial production volumes reach up to 2000 L, therefore it is possible to produce kilograms of product in a batch process kilograms (Jesus, Wurm 2011). Due to the high complexity of mammalian metabolism, holistic approaches to characterize and quantify mammalian systems are a lot more complicated than for bacterial strains (Wuest et al. 2012). Similarly, the mammalian production reveals a higher degree in biological variability therefore also the product typically reveals a certain degree of variability.

Mammalian systems are almost exclusively employed by the pharmaceutical industry for high-value products or as test systems in R&D, quality control and for safety testings but are of little importance within other industrial branches. Therefore, mammalian expression systems will not be discussed in further detail within this report.

3.2 Cell-free production systems

Biotransformations using intact cells are often difficult to control as the cellular metabolism is highly complex leading often to the formation of unwanted by-products, or further metabolisation or degradation of the desired product. The concept of cell-free production systems is based on the idea that the components that are required for a given

bioconversion can be also be isolated from the cellular environment in order to perform the transformation *in vitro* independent of the cellular metabolism. Therefore, theoretically the production process can be more economically by maximizing the utilization of the available substrates and energy.

Generally, two types of cell-free production systems exist (Aichinger et al. 2016):

- **Synthetic enzymatic metabolic pathways**: In this setting all components of the reaction environment are added individually. Typically, this involves one or several enzymes which are either isolated from their natural hosts or expressed heterologously as well as further organic and inorganic components that are required for the reaction. This type of enzymatic conversions has been well established at industrial scale for various purposes. Some relevant examples are the production of chiral fine and specialty chemicals, various applications in the food and feed industry including starch hydrolysis and production of high fructose corn syrup, or enzymatic treatments of animals feed (Vandamme, Soetaert 2010; Aichinger et al. 2016). At lab scale, complex in-vitro reactions involving up to 12 enzymes have been demonstrated to yield close to complete substrate conversions (Dudley et al. 2015; Korman et al. 2014; Chen, Zeng 2016). Moreover, non-natural enzymatic reactions have been performed in-vitro (Siegel et al. 2010; Lalonde 2016). However, the industrial application of complex enzyme cascades or even the establishment of artificial metabolic networks still face many challenges including enzyme costs, enzyme stability, engineering of novel enzyme functions, regeneration of co-factors and spatial-temporal control over biocatalyst activities (Schmidt-Dannert, Lopez-Gallego 2016; Chen, Zeng 2016). Therefore, major R&D efforts will be necessary before synthetic production platform will reach a competitive level of performance for industrial production processes.

- **Production platforms based on crude cell extracts**. An alternative approach to cell-free bioproduction is the use of complex cell extracts. In this approach the cell walls and membranes of living cells are destroyed and the lysate including the cell's biosynthetic machinery is recovered. Typically, the lysates are obtained from genetically optimized organisms and supplemented with additional enzymes and components. These can be included enzymes capable of performing non-natural reactions or non-natural molecule like non-canonical amino acids to be integrated into the resulting product. Cellular extracts combine many advantages of cell-based and synthetic production systems including a high degree of freedom to manipulate the system while taking advantage of the complete metabolic networks provided by the cell. Therefore also complex protein

modifications like glycosylsations can be synthesized in vitro. In-vitro protein translation systems are routinely applied for R&D purposes and have been commercialized by protein service providers. An industrial scale cell-free production process has been demonstrated in the past (Zawada et al. 2011) by the US-based company “SUTRO Biopharma” and the first cell-free produced biopharmaceuticals might to enter into clinical development within the next few years.2

Generally, cell-free systems offer great advantages for industrial processes since important limitations of cellular systems can be overcome. As cell-free systems are so called “open systems” these can be manipulated directly, for example process conditions can be controlled and there is direct access to the product. Therefore, cell-free processes could – in theory – run for a very long time while the product and inhibitory side products are continuously removed from the reaction mix and substrates are replaced.

A major remaining hurdle is the need for highly robust enzymes which will function over a long period of time, and which are available at high quantities at low price. Another unsolved challenge which requires further R&D investments is the issue of cofactor regeneration.

A further highly promising future opportunity for in-vitro production systems are concurrent reactions with chemical metal catalysts (Kohler, Turner 2015). While metal and bio-catalysis were long considered to be mutually exclusive due to very different requirements to reaction conditions recent advancements in enzyme engineering (see section 4) are providing solutions to this challenge. Perspectively, concurrent reaction systems promise to reduce production costs as additional purification steps between the different conversion steps could be omitted. Moreover, new reactions could become possible. However, given that this field of application is only emerging, further R&D efforts are needed before reaching a maturity level for commercial application.

2 http://www.sutrobio.com/sutro-marks-important-progress-receives-two-milestone-payments-from-celgene/. Retrieved on September 13, 2016

3.3 Innovation Potentials and Future Trends for IB production systems

As presented above, there are numerous potential production platforms for industrial biotechnology and new ones are emerging like anaerobic microorganisms and microbial consortia. The spectrum of biological functions provided by these has not yet been exploited by IB. However, major R&D investments will be necessary, from fundamental research to process engineering for these production systems to reach technological maturity for industrial application. Moreover, the choice of a suitable production platform does not only depend on the technological performance of a given platform but also on numerous framework conditions. Therefore, production yield is only one of many criteria that apply.

The exploitation of alternative **carbon sources**, in particular also those that are derived from waste streams is one of the major challenges for industrial biotechnology for the future years. Thus, robust, long-living and affordable production systems will be needed that are able to tolerate a certain degree of variability or the presence of impurities. This becomes of particular importance with respect to the valorization of waste streams.

In the last years the abilities to transfer selected functions from one organism to the other and to design powerful cell factories have grown immensely due to the recent development of powerful **tools** for bioinformatics, gene synthesis, genetic engineering, high-throughput screening and characterization (see section 4). This provides highly promising opportunities for IB to broaden the application spectrum of the existing production platform and to overcome intrinsic limitations of a given system.

However, the potential of the platforms presented above can only be exploited if they can be successfully transferred from the laboratory bench into an industrial process. Moreover, for the user major investments need to be made before adopting a novel production system: Not only is it necessary to set-up a new infrastructure which fulfils the requirements of the production system, but also to obtain expertise in a new field and potentially have to go through further approval processes by regulatory authorities. Therefore, even if the technological challenges can be met in the future, numerous non-technological aspects will greatly influence whether the IB sector will eventually benefit from these opportunities.

4 Tools and approaches for improving bioproduction processes

4.1 Screening for new enzymes and microorganisms

The biocatalyst, i.e. the microorganism or enzyme employed in a biotechnological production process, is a key component of the whole process. At present, all industrial production process are built on metabolic conversions catalyzed by enzymes or organisms that were originally isolated from nature and have been optimized for industrial applications (Aichinger et al. 2016). Typically, efforts to identify new or improve existing biocatalysts aim at the following goals:

- Increased robustness of the biocatalysts, i.e. biocatalysts that exhibit high activity even in the presenct of unfavourable conditions (i.e. the presence of solvents, high salt concentrations etc)
- Exploitation of yet unused substrates and carbon sources (i.e. carbon dioxide, lignocelluloses, raw glycerol, or waste water)
- Increasing yield, i.e. eliminating feed-back mechanisms
- Increased product specificity
- Increased conversion rate
- Alteration of the genetic or amino acid sequence to a degree that the resulting gene or enzyme is no longer covered by IP protection without compromising on the features of the production organism or enzyme.

Nature provides an enormous diversity of microorganisms and enzymes, of which only minimal fractions are known and can be cultivated for industrial applications. Various approaches have been developed in order to identify and isolated individual organisms or enzymes from large collections. Perequisite for any screening approach are the availability of an assay to test for a desired feature as well as means to isolated individual candidates that exhibit the desired.

Generally, there are two opposing concepts for the **optimization of a biocatalyst** which are also often used in combination with each other:

- Rational design and optimization: This requires profound knowledge of the organism or enzyme to be optimized including sequence information and data on protein structure as well as on the limiting factors. Typically, rational optimization is based on computational modeling. For example, modeling of enzyme

structures will allow for predictions on individual amino acids as targets for enzyme optimizations (Kries et al. 2013). Metabolic modeling is used to identify target genes for strain optimizations in organisms trait (Zhuang, Herrgard 2015).

- Random screening: In this approach a random collection is screened for suitable candidates. This collection can either be a natural isolated or a mutation library of given gene. In a subsequent screening, individuals with improved characteristics are identified and isolated. Therefore, knowledge on the regulatory mechanisms or coding sequence is not necessarily required.

In the past years many concepts for **enzyme discovery and optimization** have been developed, including many which combine both rational and random approaches (Bornscheuer et al. 2012). The development of ultra-high-throughput approaches based on microfluidic sorting systems that allow to screen libraries containing several million individuals within a few hours as well as an improved data base enabling better predictions are considered important drivers for the development and optimization of biocatalysts (Choi et al. 2015; Bornscheuer et al. 2012; Dörr et al. 2016; Zinchenko et al. 2014). A general limitation of high-throughput screening approaches is that very often no suitable assays are available which are compatible with high-throughput systems and that real process conditions cannot be mimicked well. Therefore, even though candidate may exhibit high performance under test conditions this may not be transferable onto process conditions. Due to the high costs associated with the optimization of production organisms and industrial enzymes this represents an important innovation hurdle. Therefore, the development of improved test and screening systems that can be employed also for ultra-high throughput settings or novel approaches for biocatalyst discovery and optimization could be a great driver to access yet unused enzymes and organisms for technical applications.

In the past years, metagenomic approaches have gained importance (Ferrer et al. 2016). The identification of enzyme sequences from environmental genomes (i.e. metagenomes which represent the genetic information of a whole environmental population) bears great potential since also novel sequences from unculturalbe can be identified.

However, despite the fact that there are many academic reports on the improvement of enzymes or production organisms there are only very few examples which have been translated into an industrial process The number of **commercial enzymes** listed by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) has only risen by approximately 50 from ca. 200 to ca. 250 entries in the past decade (Nusser et al. 2007; Aichinger et al. 2016), despite the enormous growth in the amount of se-

quence data available. One outstanding example was the development of a novel transaminase in a joint venture by Merck and Codexis for the manufacture of sitagliptin, the active ingredient of a type 2 diabetes drug (Savile et al. 2010). According to experts, the high investments in terms of time and money that are typically needed present a major hurdle.

4.2 Genetic engineering and gene editing

Virtually all organisms and enzymes that are used in industrial process have resulted from genetic optimizations. These can be achieved either by random mutagenesis and screening or by directed genetic manipulations.

Random mutations occur naturally or can be introduced, i.e. through chemicals or radiation. These treatments result in mutations that are spread statistically over the whole genome. Therefore, in order to obtain a mutation in a target region, usually a high number of mutations has to be introduced into the genome. Thus, beside the screening efforts also exhaustive analysis and backcrossing are needed in order to remove unwanted effects. Typically several rounds of selection and optimization are needed in order to obtain an organism with the desired characteristics.

With the advent of gene technology, **site-specific manipulations** of genes and genomes have become possible. These include the alteration of individual genes, the deletion of complete sequences as well as the insertion of genetic sequences into the genome.

Several techniques have been developed in that enable the alteration of genomes. Typically, these techniques have been optimized for a given species. For most highly studied model organisms like E.coli or S.cerevisiae a large set of tools for genetic modifications and strain collections have become available allowing for site-directed manipulations and directed insertions of novel sequences whereas the vast majority of organisms were not as easily accessible to directed genetic manipulations.

The development of **gene editing** (or genome editing) technology is presently revolutionizing genetic engineering. This technology based on programmable nucleases, most importantly the CRISPR/cas9 system (Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas)9 system), allows for the introduction of targeted, tailored changes into the genome. Since it was first reported in 2012 by Jinek and co-workers (Jinek et al. 2012), gene editing based on the CRISPR/cas9 system has been applied to a large range of organisms including micro-

organisms, insects, plant and animal species (Hsu et al. 2014; Doudna, Charpentier 2014). Most importantly it was also applied in species in which precision genetic alterations have not been possible before.

The impact of gene editing technologies has widely been discussed in the field of human health and genetically modified plants. However, also for the field of IB gene editing is of particular importance in several ways (Jakociunas et al. 2016; Barrangou, van Pijkeren 2016; Selle, Barrangou 2015; Deutsche Forschungsgemeinschaft et al. 2015):

- Due to its ease of application genetic manipulations can be achieved at significantly lower costs than before.
- Multiple genetic alterations can be introduced into a genome at the same time.
- It enables site-directed genetic manipulations also in industrial relevant strains that could not be accessed by genetic engineering previously.
- Genetic modifications can be introduced at single nuclear base level. Moreover it is possible to remove foreign genetic sequences and to modify genomes without inserting foreign DNA. Therefore it becomes possible to design strains that do not carry antibiotic resistance markers and cannot be distinguished from naturally occurring variants.

Therefore, gene editing is believed to hold a great potential to increase the efficiency of industrial strain improvement and have far- reaching applications.

However, due to the fact that gene editing technologies have only been developed a few years ago and many open questions remain. One of the most pressing is the question of off-target effects: Even though the CRISPR/cas systems enables to target genomes in much more specific way than other techniques that were available before it cannot be ruled out that also non-targeted sites in the genome are affected with unknown consequences. Therefore further research and improvement of gene editing tools are required.

In IB gene editing has mainly been applied for metabolic engineering of **eukaryotic systems** like yeast (Generoso et al. 2016; Stovicek et al. 2015) whereas the applications in prokaryotic organism lacks behind. A major obstacle is the fact that nuclease-based gene editing systems like CRISPR/cas9 introduce double-strand breaks in the genome which are lethal in prokaryotes due to a lack of a suitable repair system (Mougiakos et al. 2016). Another reason why gene editing tools are not taken up as quickly for prokaryotes could be the fact that a highly developed tool box for most industrially relevant microorganisms exists already. Therefore, at present gene editing

tools are not discussed as intensively as in other fields like genetic engineering of plants or human health contexts. In the field of IB, gene editing has mainly been applied in eukaryotic systems like yeast but further efforts to adjust gene editing tools to procarytic systems are needed in order to use its enormous potential for the manipulation of prokaryotic hosts in the future (Pul et al. 2016; Mougiakos et al. 2016)

4.3 Metabolic engineering and Synthetic Biology:

The development of numerous techniques for genetic manipulations and a quickly growing knowledge base have made it possible to reprogram cellular metabolism in order to enhance the production of native metabolites or to enable cells to produce new products (Nielsen, Keasling 2016).

The development of an engineered E.coli strain which produces the chemical building block 1,3-propanediol in a joint venture by DuPont and Tate&Lyle is considered a milestone in metabolic engineering as it was the **first commercial example** were a complete foreign metabolic pathway was introduced into a novel host. However, despite the enormous progress in the understanding of cellular metabolism from –omics approaches in the past years and novel tools for genetic manipulations, the development of a commercial production strain is still a length and laborious process which usually requires numerous rounds of strain construction and improvement (Nielsen, Keasling 2016). Major obstacles to more rational approaches for metabolic engineering are the high complexity and robustness of cellular metabolism, the existence of genetic redundancies and regulatory mechanisms which still have to become elucidated for the vision of cell factories to become realized. In this vision, tailor-made cell factories are employed for the cost-efficient production of fuels, chemicals, food and feed.

Undoubtedly, bioinformatic **modeling of cellular metabolism and simulations** are essential tools for rational approaches but more knowledge is needed on the cellular regulatory mechanisms. For example more basic research will be needing in order to elucidate the roles of regulatory non-coding RNAs, how mRNA translation is regulated and which further post-translational regulatory mechanisms exist (Wu, Jaffrey 2016; Robinson et al. 2016) and in order to develop strategies how these mechanisms can be controlled. Moreover, a broad database will be needed in order to develop robust and reliable models that will allow for the simulation and design of production organisms.

Another major hurdle is the fact that at present there is the **lack of standardization**, i.e. a set of standardized conditions or model organisms and strains. As a result, numerous efforts are undertaken in parallel and with a high degree of redundancy but it is

often not possible to access data, extrapolate or transfer findings onto different settings. Thus, significant opportunities for more efficient value-adding for R&D activity could arise if the scientific community would define and agree on a set of standards for the production of data and for the data management.

The concept of **synthetic biology** goes beyond the idea of metabolic engineering (Lorenzo 2010; Sauter et al. 2015). Instead of only optimizing a given pathway in a production organisms and transferring individual functions that synthetic biology aims at the reduction of cellular functions to create a minimal cell factory. There are two general approaches in synthetic biology:

- The top-down approach aims a the reduction of genomes to the minimal functions which are required for the maintenance of life.
- In a bottom-up approach synthetic biologist aim to assemble biomimetic structures in order to specifically adapt newly designed structures to the relevant requirement.

Important drivers for synthetic biology have been the development of novel tools for genetic engineering, next-generation sequencing and the exponentially improving DNA synthesis capabilities but there still is an urgent need to develop enabling technologies and tools and to achieve a more fundamental understanding of biological systems and how these can be designed and controlled.

Several methods for genome reductions have been developed recently (Xue et al. 2015; Martinez-Garcia, Lorenzo 2016) and the number of host with reduced genomes is growing rapidly but at present major technical hurdles remain. These require more fundamental research in order to increase the understanding of biological systems and to develop means how it can be controlled (Martinez-Garcia, Lorenzo 2016).

Despite the clear focus of synthetic biology on industrial applications there are only a **few successful examples** which have reached a commercialization level. Like metabolic engineering also synthetic biology often struggles with the challenge of the successful transfer laboratory finding into commercial applications, in particular the scale-up of a laboratory process to commercial volumes (Chubukov et al. 2016). Beside technical hurdles, synthetic biology is also facing public concerns which represent an additional obstacle for industrial applications.

All in all, there is a broad consent among stakeholders that synthetic biology bears a great potential as an enabling technology to achieve the long-term vision of a sustainable, industrial biotechnology.

4.4 Systems Biology and Bioinformatics

Systems and bioinformatics' approaches have had a major impact on IB in the past years. A rapidly growing number of parameters is routinely determined and analyzed enabling the characterization of biological systems by an array of quantifiable variables (i.e. genome, transcriptome and proteome data, metabolite composition, interaction data). The database for modeling and prediction is growing continuously. And virtually all fields of IB, from the design of the biocatalyst to process monitoring, have adopted data-driven and systemic approaches (Becker, Wittmann 2015, 2016; Nielsen, Keasling 2016; Borodina, Nielsen 2014; Burk, van Dien 2016; Chubukov et al. 2016). Therefore, systems biotechnology and bioinformatics must be considered key enablers for IB.

Despite the wide adoption of these approaches and a general agreement on their importance among the scientific community, there are still major **hurdles** which prevent an efficient use of the data which is available (BioÖkonomieRat 2012):

- A **lack of standardization** for data collection and management: Due to the high complexity of biological systems it is usually not possible to use data from one experimental setting to make valuable predictions for another experimental setting. Thus, there is a need to define standards and establish data management and collection systems which improve access to data as well as comparability and transferability of data. Moreover, common formats for data storage are needed which allow exchange and comparison of datasets between databases.
- **Insufficient interaction between biological sciences and computer sciences**: As expert knowledge from two disciplines are needed, specific expertise at the interface of the two is needed.
- **Efficient tools for data processing**: As the amount of data is growing rapidly so is the understanding of the complexity of biological systems. The development of bioinformatic tools to process and analyze these data require for interdisciplinary competence both in informatics and biological understanding. As end users of biological data are typically not informatics experts also user-friendly tools for data mining and visualization of data are needed.
- The utilization of **computer capacities** must be optimized across institutions in order to allow for fast and efficient access. New cloud based concepts might be helpful to this end.

- International **framework conditions for exchanging and using data internationally** and across institutions must be defined. Beyond legal questions and ownership, this also includes standards for sage data storage.

4.5 Bio-electrochemical systems

Bioelectrochemical systems (BES) have gained much attention in the past years. The general concept behind BES is the idea to utilize the capacity of many microorganisms or enzymes to transfer electrons. In general, there are two concepts for bio-electrochemical systems:

- Bioelectrochemical production or bioelectrocatalysis in which the biosynthesis of fuels or chemicals is driven by electrical energy (Harnisch et al. 2015)
- Bioelectrolysis in which microorganisms or enzymes are used for the production of an electrical current or the formation of hydrogen (Escapa et al. 2016).

A great potential BES is seen in the context of waste treatment since it could enable the utilization of biodegradable waste for the formation of hydrogen (H_2), biofuels, or other value-added products (Lu, Ren 2016).

A first economic analysis by Harnisch and co-workers indicated that BES using electrical energy for driving bioproduction of lysine could in theory be more economical than using sucrose an energy source (Harnisch et al. 2015).

At present there are still fundamental gaps in the scientific understanding of electron transfer between biological systems and artificial electrodes. For a large variety of microorganisms the capacity to act as electron donors or acceptors has been shown, making them potential candidates to be used in BES. In addition, numerous feasibility studies have been performed at lab scale (Kracke et al. 2015). But so far it has not been determined which organisms provide the most promising characteristics for their utilization in BES. The systems that have been developed so far still lack efficiency and face severe limitations with respect to their up-scaling capacities (Butti et al. 2016). Thus, major technological hurdles still have to be overcome before bioelectrochemical systems will reach a maturity level for industrial applications.

4.6 Innovation Potentials and Future Trends for Tools and Approaches for the development of novel bioprocess systems

Many highly exciting tools and technologies have been developed in the past years which are often claimed to have the potential to revolutionize IB. In several interviews experts indicated that these new technological opportunities and the strong scientific base and expertise which was present in Europe presented important opportunities for advancing IB in the future. Knowledge-based approaches are entering all fields of biotechnology but it happens at a different speed in each field. While some areas of IB which have traditionally been more closely associated with life sciences than engineering (i.e. the enzyme industry) are typically implementing new scientific trends more rapidly. For other fields that are typically associated with engineering rather than life sciences (for example waste management but also food and feed production) it may be more difficult to adopt novel approaches. Within the latter typically natural biological systems are employed which have not been characterized in detail by systems biology approaches. Therefore, these fields could benefit greatly from the novel opportunities provided by systems biology and bioinformatics. However, this will require dedicated efforts to bring together disciplines which until now do not have very many points of interaction.

5 Exploitation of novel feedstocks

Classically, microbial production of chemicals relies on feed stocks rich in fermentable sugars like corn starch, sugars, or molasses. As these are typically derived from sustainable, non-fossil origins, industrial biotechnology is considered a key enabling technology for the bioeconomy. However, these classical feedstocks are typically derived from edible parts of plants, which raises the question of how to distribute feedstocks between foot, feed, and fuel applications. Great efforts are being taken globally to exploit novel non-food resources for industrial applications. This represents both a great challenge and a great opportunity for industrial biotechnology. At present there are only few examples of commercialized bio-processes based on alternative carbon sources whereas the vast majority relies on classical carbon sources based on starch or fermentable sugars (Gustavsson, Lee 2016). Nevertheless, there it is widely agreed on in the scientific community that the valorization of alternative feedstocks represents an important opportunity for IB in Europe.

In the following, some of the most important alternative carbon and energy source for IB are described.

5.1 Lignocellulose

Lignocellulose is a heteropolymer abundantly present many terrestrial plants providing them with strength and rigidity and protecting them against microbial attacks. Lignocellulose is composed of cellulose (30-60% of total feedstock dry matter), hemicelluloses (20-40% of total feedstock dry matter) and lignin (15-25% of total feedstock dry matter). Lignocellulose makes up a large portion of the non-edible parts of plants which are often not valorized during agricultural production. This makes the utilization of lignocelluloses as a feedstock for industrial production highly desirable. Lignocellulosic biomass is highly abundant in Europe (i.e. wood, grass, or straw). But its conversion is very challenging due to its heterogeneous composition of various sugars (including hexoses and pentoses) and aromatic compounds and the fact that lignocellulose is highly stable and resistant to biological degradation. At present, lignocellulosic biomass is mainly used thermally, but techno-economic studies indicate that it will be necessary also valorize the lignocelluloses fractions from biomass in order realilze the vision of bioeconomy (Narron et al. 2016).

Due to its intrinsic heterogeneity and high resistance to microbial conversions lignocellulose equires extensive **pretreatments and fractionations** before it can be transformed into valuable products (Beckham et al. 2016). Several methods for pretreatment and fractionation of streams have been developed. Typically, these are based on ther-

mal and/or chemical hydrolysis and depolymerization and therefore require high amounts of energy and/or chemicals. Beside the high costs associated with pretreatment a further barrier is the fact that the resulting material streams can usually not be used directly for subsequent microbial or enzymatic conversions due to the presence acids or organic solvents in the stream. Therefore, in order to establish a sustainable and economic process for the valorization of lignocelluloses, pretreatment technologies will be required that are compatible with biotechnological conversions. This will require both, the development of pretreatment and fractionation processes that use milder conditions, and the development of robust biocatalysts. Moreover, the area of lignocelluloses utilization could greatly benefit from the attempts to couple chemical and biological catalysis (Schwartz et al. 2016) (see also section 3.2). There are attempts to develop biological treatments for lignocellulosic materials in which bacteria, fungi, or enzymes are used in order to circumvent some of the disadvantages of chemical and thermal processes. However, biological pretreatment is not expected to become competitive in the near future due to the slow rates of conversion and high enzyme costs (Liguori, Faraco 2016).

Mainly due to intensive public funding, several **pilot plants** that produce biofuels or chemicals from lignocellulosic feedstocks have been installed in European countries, therefore with respect to its scientific and technological capabilities as well as the availability of feedstocks Europe is in a favorable position. Remaining technological barriers for large scale utilization of lignocellulose biomass are the high costs associated with pretreatment and high costs for enzymes (E4tech et al. 2015).

Even though lignocellulose is mainly used for the synthesis of biofuels at the moment several stakeholders have pointed out that the valorization of lignocelluloses could present an important opportunity for Europe in particular if processes can be developed which will allow valorizing the intrinsic functionalities of lignocellulosic materials.

5.2 Carbon dioxide

Due to the fact that excessive carbon dioxide exhaustions are seen as a major cause for global warming the utilization of CO_2 as a feed stock seems highly desirable. However, chemical processing for CO_2 is technically very challenging due to the fact that CO_2 is an end production of respiration and is therefore chemically inert. Significant amounts of energy are required in order to reduce CO_2 to carbon monoxide which can serve as building block or precursor for the synthesis of polymers, biofuels or chemicals. Given that several biological systems are naturally using CO_2 as a carbon source

to build higher carbon compounds like sugars biotechnological reduction of CO_2 seems to bear a great potential. Several biotechnological approaches for the utilization of carbon dioxide have been developed (ElMekawy et al. 2016):

- **Photosynthesis based conversion**: As green plants, microalgae are photoautotrophs that use sunlight to build sugars and other metabolites from atmospheric CO_2. Several pilot plants that employ algal technologies to reduce CO_2 content in flue gases or from the atmosphere have been set up in the past years. Algae are of interest for numerous biotechnological products (ranging from biomass to algal biofuels) (Aichinger et al. 2016; Cheah et al. 2016).
- **Microbial carbon capture**: Under certain anaerobic conditions some methanogenic bacteria are capable of utilizing carbon dioxide and hydrogen to produce methane (Goyal et al. 2015; Hara et al. 2013). However, due to high technical challenges these approaches are still far from reaching technological readiness to for industrial applications.
- **Enzymatic carbon capture** (Xia et al. 2014; Claren et al. 2009). Some enzyme have been shown to be able to catalyze the fixation of atmospheric CO_2 including carbon anhydrases, and isocitrate dehydrogenases. In particular immobilized carbon anhydrase was suggested as a promising approach as it is potentially competitive with non-biologic approaches for CO_2 fixation, but enzyme stability and life time remain limiting factors (Zhao et al. 2016)

All in all, there are several very interesting biotechnological approaches for the utilization of CO_2 as carbon source. However, at present it seems unlikely that any of these technologies will reach technological maturity levels that will allow for large scale valorization of CO_2 within the next decade. Beside the fact that the existing conversion technologies do not yet reach sufficiently high performance levels another challenge will be to integrate the resulting intermediate product into a subsequent production process. Beside biotechnological approaches for carbon capture also chemical approaches have been suggested and are also being investigated intensively. One example is the “dream production” which was developed by Covestro (formerly Bayer Material Science). In this process polyurethanes (which are used for the production of industrial foams) are synthesized from CO_2.Covestro has announced to commence operation of a production line for 2016.[3] Thus, at the moment it questionable whether and to which degree biotechnological approaches will contribute the material valorization of CO_2.

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3 http://www.covestro.de/en/projects-and-cooperations/co2-project. Last accessed on October 22nd, 2016.

5.3 Glycerol

Glycerol makes up the chemical backbone of oils and fats. Recently, biofuels like biodiesel and bioethanol have become important sources for crude glycerol which is formed as a stoichiometric by-product, making up about 10 % by weight in biodiesel production. Therefore, as a **side effect of increased biodiesel production** huge amounts of raw glycerol have become available which exceed by far its demand: In 2015 approximately 11 Mt of biodiesel were consumed in the European Union (EUROBSERV'ER), the production of which has been accompanied by the formation of more than a million t of crude glycerol. Many microorganisms are capable of using glycerol as a carbon and energy or have been genetically modified to do so. These include also industrially relevant bacterial and yeast strains like *E.coli*, *S.cerevisiae*, *C. glutanicum*, and *B.subtilis* (Wendisch et al. 2016).

Several interesting **products** have been produced from glycerol as a feedstock including 1,3-propanediol, 1,2-propanediol, succinic acid, propionic acid, ethanol, buthanol as well as several amino acids (Aichinger et al. 2016; Wendisch et al. 2016; Meiswinkel et al. 2013). However, the utilization of crude glycerol as a waste product from biodiesel production is challenging due to the presence of multiple impurities like residual methanol, free fatty acids and high salt concentration. Since the purification of crude glycerol is costly, large scale glycerol valorization will only be economical if robust production platforms for commercial scale production will become available. First academic approaches exist but these have not yet been implemented at industrial scale (Meiswinkel et al. 2013; Yang et al. 2012; Ashby et al. 2011; Wischral et al. 2016). Beside these technological barriers, the role of glycerol will eventually depend on the question whether or not cheap glycerol will be available from biodiesel production in the future or if alternative transportation concepts will become dominant in the future.

5.4 Waste streams

Recently, waste streams have gained much attention as potential carbon and energy source for IB. While waste water treatment is a traditional field for application for biotechnology the valorization of waste streams for the production of biobased materials and energy is currently receiving much interest within attempts to enable a **circular and low carbon bioeconomy** (Mohan et al. 2016).

At present, waste streams are almost exclusively used for the production of biofuels and biogas but a great opportunity is seen in the production of sustainable chemicals from waste streams (BIO-TIC 2015a). Beside urban waste, many industrial waste

streams are rich in organic compounds and therefore theoretically suitable as feed stocks for fermentative production processes. Major challenges are the facts that waste streams typically contain high amounts of toxic substances and vary in their compositions. Therefore, highly robust and flexible production systems are needed.

The utilization of **microbial consortia** appears to be a promising approach. Microbial consortia have been employed traditionally in waste water treatment but have received rather little attention from systems biology and metabolic engineering (see also sections 3.1.1.1 and 4.4). Therefore, the development of tools to control and engineer microbial communities could be an important driver for the valorization of waste streams (Song et al. 2014).

Further technological approaches that might have a great impact on the utilization of waste streams in IB are microbial bioelectrolysis (Harnisch et al. 2015; Lu et al. 2015; Dopson et al. 2016) and anaerobic fermentation (Sawatdeenarunat et al. 2016).

All in all, the valorization of waste stream is a highly promising opportunity but major technological hurdles and gaps remain which have to be overcome to allow for the establishment of an industrial scale process.

5.5 Methane

Methane, CH_4, is the main compound of natural gas and biogas and is abundantly available. Therefore, it has received much attention as potential feedstock for the production of biofuels and bio-based chemicals (Ge et al. 2014; Strong et al. 2015; Haynes, Gonzalez 2014; Haider et al. 2015; Schrader et al. 2009). Even though there are several scientific reports on biotechnological applications based on methanotrophs at present these have not reached a maturity level which would allow for industrial exploitation of this technology. A technical challenge is also the fact that methane and oxygen can form explosive mixtures, therefore anaerobic processes would be desirable.

5.6 Innovation potential and future research topics for the valorization of alternative feedstocks

The utilization of alternative feedstock is an enormous challenge since this typically requires the establishment of a completely new production process including novel production organisms, process technology and process design.

Fossil and alternative feedstocks like plant materials or waste streams also differ from each other with respect to their chemical functionalities: While fossil feedstocks are mainly composed of hydrocarbons alternative feedstocks typically show a heterogenous composition and carry a broad spectrum of chemical functions including hydroxyl groups and aromatic compounds. The present applications based on alternative feedstock mainly yield **drop-in products** (i.e. products that are identical to those that are normally made from fossil feedstocks). This is often desirable since these products can feed into existing value chains. However, drop-in solutions will always have to face direct competition with their fossil-based counterparts. Despite the fact that fossil resources are declining it is highly unlikely that bio-based products will be able to compete in economical terms.

Therefore, also **new products** will have to be identified that can be produced from alternative feedstocks and which have specific characteristics that cannot be achieved from fossil resources. Thus, in order to benefit from high value-added from novel feedstocks requires also openness to alternative synthesis routes: Instead of introducing functionalities onto a hydrocarbon chemists and biotechnologists will have to find efficient ways how to utilize existing functionalities and to remove unwanted functions. On the one hand this is a major technical challenge, but at the same time this represents an important opportunity since the synthesis of novel chemicals and materials will be possible. However, it will be a challenge for R&D in IB to determine which materials and functionalities will provide benefits and meet the end uses' demands and to find ways to provide these at an industrial scale.

Thus the utilization of alternative carbon sources is facing challenges which go far beyond technological developments and will have far reaching effects on various levels of the value chain including product range but also aspects like feedstock supply, transportation and logistics.

6 Bioprocess Engineering

6.1 Process development and optimization

In the field of IB, the successful establishment of a production process is one of the biggest challenges during the innovation process. The bioconversion step is typically developed in laboratory background under highly controllable conditions and has then to be scaled up to an **industrial production plant** with less controlled condition. In the industrial setting often further aspects beyond productivity become highly important, for example economical performance, but also workflows, IP issues, and regulatory requirements which have to be met. Therefore, the design of a biotechnological production process goes far beyond the optimization of the individual technologies applied in each step. Instead an integrative view covering the production process is required which takes into account the whole process as well as the framing conditions of the whole value chain (Weiss 2016; Gustavsson, Lee 2016).

A classical bioprocess consists for three subsequent steps:

- Up-stream processing (USP) which comprises all steps that lie ahead of the actual bioconversion. Important aspects of the USP are supply, logistics, storage and pretreatment of feedstocks, the supply and storage of the biocatalyst, and all preparatory measure required for the bioprocess (for example cleaning and, sterilization of the bioreactors).

- The bioproduction process: In the actual bioproduction process the substrate is converted to the desired product.

- Downstream processing (DSP): This includes all steps after the actual bioconversion. Important aspects are product recovery and removal and potential valorization of side-products, further product processing, the recovery of the biocatalyst, and waste management and valorization.

Depending on the desired application, specific **bioreactor formats** have been developed. Stirred-tank bioreactors which are operated in either batch or fed-batch mode are the most import type of bioreactor for industrial biotechnology (Aichinger et al. 2016). The bioreactor will have to fulfill the needs of the production system; therefore novel production platform may require new concepts, for example tubular photobioreactors which have been developed for the cultivation of photoautotrophic organisms like microalgae. Therefore the development of novel bioproduction systems always goes along with the adaption of the existing cultivation systems or the development of new concepts for their cultivation. Bioprocess engineering has already greatly benefited

from the data systems biology approaches and will continue to do so. The characterization of a bioprocess also at the metabolic level will provides deep insights into the dynamics of these processes and allow for the development of adequate means to control these.

A great potential is also seen in the establishment of **continuous production processes** which would have the potential to greatly reduce operating costs (BIO-TIC 2015a). At present continuous production systems are only applied in in-vitro systems but at present continuous fermentation systems are still lacking (BIO-TIC 2015b).

Moreover, the implementation of large scale bioproduction facilities will be accompanied by novel challenges for the waste and water management. While chemical production typically takes place at high concentration, waste water streams from biotechnological productions are typically highly dilute and require different treatments than waste streams from the chemical industry. Therefore both is needed, the development of water-efficient bioprocesses and novel concepts for treating IB waste streams.

All steps of the bioprocess should be taken into account during the design of **optimization of a bioprocess**. For examples, the optimization of a biocatalyst must take place as an integrated effort to develop a complete bioproduction process (including concepts for USP and DSP). However, while such an integrative approach was highly desirable, very often the processes of USP, bioconversion, and DSP are developed and optimized independently from each other at lab scale which often result in incompatibilities and difficulties during the scale-up process (BIO-TIC 2015a). High levels of interdisciplinary expertise required in bioprocess engineering remain a major challenge despite the fact that bioengineering has lately experienced a great shift from an engineering discipline to a highly interdisciplinary field integrating also systems biology and bioinformatic for monitoring and modeling production processes. In particular, specific scale-up expertise as well as reliable predictive scale-up models are needed (BIO-TIC 2015a). As a prerequisite for these it will be necessary to extend the information base on bioprocesses, enable access to data and to establish common standards.

Ideally, bioprocess design and optimization is done in an integrative approach which takes into consideration not only all steps of the bioproduction process but the whole value chain (Weiss 2016).

6.2 Integrated biorefineries

The concept of an integrated biorefinery goes far beyond isolated bioproduction processes. It involves the integral processing of biomass (or waste streams) into a broad range of products such as value added chemicals and materials, in addition to bioenergy and biofuels in a sustainable way (Jong, Jungmeier 2015). This concept aims at the maximal valorization of all material and energy streams.

Biorefineries apply a large spectrum of technologies for processing, including mechanical, chemical, thermochemical as well as biological processing (Aichinger et al. 2016). Biological processing technologies including fungal, bacterial and enzymatic treatments are considered key enablers for the implementation of integrated biorefineries due to the fact that they typically require little energy and do not require the use of toxic chemicals (Liguori, Faraco 2016).

Biorefineries can be classified based on a variety of features, like their technological implementation status, the type of raw materials used, the main type of intermediates which are produced, or the main type of conversion process applied (Jong, Jungmeier 2015).

Conventional biorefineries based on starch and sugars crops or wood have existed for a long time, e.g. for the production of sugars, starch, bioethanol, oil, dietary fibers, pulp and paper. For these applications also edible parts from plants like corn starch and sugarcane as feedstock are frequently used thereby leading to competition with food and feed production. In order to avoid this **competition advanced biorefinery concepts** based on alternative feedstocks like whole crop, oleochemical, lignocellulosic feedstock, green, and marine biorefineries have been developed. Several of advanced biorefineries are presently being operated as pilot plants in Europe and the Americas. In addition to plant-based biorefineries also waste water biorefineries are gaining attention. At present, commercially operated biorefineries typically only produce a single product (i.e. bioethanol from sugarcane) while the remaining biomass fractions are used thermically. The vision a fully integrated biorefinery would require the valorization of all fractions obtained from biomass. In Europe several projects aiming at the establishment of pilot plants for integrated biorefineries have been initiated in the past years. Many of these are operated as public-private partnerships but major R&D investments are still required for integrated biorefineries to decrease their costs and improve performance, in order to become competitive with fossil fuels.

However, it seems highly unlikely that biorefineries will be able to compete with fossil-based production processes regarding their economic performance within the next

decades. Thus, innovative products with novel functions which can be made exclusively from biomass present an important opportunity for biorefineries in Europe.

However, despite the need for further R&D to improve technological performance of biorefineries, the **non-technological aspects** are the major obstacles for the broad implementation of biorefineries in Europe. Important factors are the availability of the feedstocks and the oil prices. A low oil price as it is being experienced at the moment (autumn 2016) is hampering the demand for bio-based products. Moreover, European regions are highly heterogeneous with respect to their technological capacities and in terms of biomass availability. Therefore, it will have to be determined individually for a given region which concept will be economic and sustainable.

6.3 Innovation potential and future trends in bioprocessing

Any scientific discovery or innovation will only be beneficial to the European biotech industry if it can be implemented in a bioprocess that meets the requirements of a commercial production process. While R&D typically yields many exciting concepts for novel bioconversions typically USP and DSP receive less attention despite the fact that they are essential for the economic performance of a bioprocess. Therefore, concepts are needed and must be implemented how to adopt an integrative and holistic view already during early phases of R&D.

In the future, biological conversions will only be one option among the technologies that can be applied in a complex production system like a biorefinery. While biocatalysts have to adapted to become compatible with more extreme process conditions which are often needed for non-biological conversions the integration of various conversion technologies also require for novel concepts for process design.

Perspectively, in order to meet the challenge of moving towards a sustainable bioeconomy these efforts will even have to go beyond specific value chains and production processes. For increasing the efficiency of IB production processes it will be necessary to identify and exploit synergies and to consolidate putatively unrelated production processes also across industrial sectors, for example by exchanging energy and substrate streams across production processes and across industrial branches (BIO-TIC 2015b).

A further non-technological challenge during for process design is to anticipate the end user's needs and demands. Value chains in IB are typically highly complex and involve numerous stakeholders. End users are typically operate outside the dedicated IB

branch and are therefore not familiar with potentials and limitations. On the other hand the IB scientific community is often not aware the end users' needs and are therefore not able to provide solutions. Private-public partnerships which also include the end users could be a possibility to improve the mutual understanding and to develop IB processes that will help to meet industrial needs.

7 Conclusion

The technological progress that was achieved in the last years has greatly enhanced the capabilities to understand biological systems and to manipulate them. Novel tools for genetic engineering, bioinformatics, systems approaches, and sequencing and DNA synthesis together are important drivers for innovation in IB. These technological capacities together with the high level of expertise which is present across Europe put the European Union in an excellent position to remain the leading region in IB. In order to benefit from these potentials R&D in IB should address the following issues:

- Increase efforts to bring together engineering and scientific disciplines and enable mutual learning to benefit from each other's expertise in order fill gaps on both sides. An example would be to link environmental engineering more closely with systems biology and metabolic engineering to enable novel concepts for waste valorization.
- Adopt an integrative view that goes beyond biotechnology. Within the concept of a sustainable bioeconomy biotechnology is only one technology which will be applied in conjunction with other technologies. Therefore, R&D should also address the question of how different processing technologies can be linked. This also requires an interdisciplinary agreement on processing standards.
- Focus on the valorization of non-food feedstocks and the development of suitable technologies. Alternative feedstocks likes waste water or lignocelluloses are different from classical feedstocks for IB in many ways, including their heterogeneous and variable composition. IB offers a great potential to valorize these feedstock if the technological challenges are addressed.
- Address the secondary challenges that go along with biotechnological production, like water-efficient process design and waste water management.
- Definition of standards and the development of predictive models which will allow for more efficient knowledge transfer.
- Enable access to data and platforms for data sharing.
- Focus on high-value products that are unparalleled in the chemical industry rather than drop-in solutions which will be unlikely to become competitive with conventional synthesis in the near future.
- Take a value chain perspective from the beginning linking research activities already in early stages to the specific requirements of industrial processes and market needs.

Furthermore, the European Union will also have to address profound non-technological challenges, including societal and economic aspects. For example, several stakeholders indicated that a lack of funding to transfer innovations from R&D settings to commercial applications (for example for the installation of pilot plants) but also societal concerns about biotechnology were important major hurdles that IB is facing in Europe.

All in all, several groups could greatly benefit from the IB R&D activities in Europe including the scientific community, the IB industry, and end users for IB products. For a maximum benefit it will be vital to provide platforms for these communities to exchange their views and to develop a mutual understanding on each other's the needs, demands, capabilities and limitations.

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