Biotechnology Advances 86 (2026) 108736

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Decoding bioprocesses with transcriptomics: current status and future potential

Nadja Alina Henke [a,*], Boas Pucker [b], Alexander Grünberger [a]

[a] Microbial Biotechnology, Microsystems in Bioprocess Engineering, Institute of Process Engineering in Life Sciences, Karlsruhe Institute of Technology, Karlsruhe, Germany

[b] Plant Biotechnology and Bioinformatics, Institute for Cellular and Molecular Botany (IZMB), University of Bonn, Bonn, Germany

ARTICLE INFO

Keywords:
Transcriptomics
RNA-seq
Bioprocess engineering

ABSTRACT

Transcriptomic analyses represent widely used state-of-the art methodologies in molecular biosciences going back to the early 1960s. Over the last years, transcriptomics has become increasingly important in the field of bioprocess engineering. Systematic transcriptomics platform technologies (especially RNA-seq) play an accelerating role to investigate the gene expression profiles of cells in diverse bioprocesses, covering prokaryotic and eukaryotic cell systems. This review summarizes how different transcriptomics methodologies from RT-qPCR to microarrays and RNA-seq have been applied in bioprocess engineering to date. The major scopes of the reviewed works can be categorized as the following: strain/cell line characterization, investigation of culture/media conditions, process operations as well as scale-up/down studies. Subsequently, a perspective is given how emerging sequencing-based transcriptomics could envision the understanding of population diversities in space and time aided by single cell analysis (scRNA-seq) as well as transcriptional histories (Record-Seq).

1. Introduction

1.1. Bioprocess engineering as basis for more sustainability

The economic transformation into a bioeconomy has been anchored in the 17 Sustainable Development Goals (SDGs) of the United Nations (UN General, 2015). The political circumstances in the context of the climate and energy transition inevitably lead to an enormous challenge in the field of bioprocess engineering. In the coming decades numerous bio-based alternatives to petrochemical processes have to be established. Substitution of fossil raw materials can be achieved by biotechnological strategies such as fermentation (Cankar et al., 2023) and biocatalysis (Miller et al., 2022). It can be assumed that many industrial processes have to be developed in the context of the bio-based industry. Bioprocess engineering addresses both bioprocess development and scale-up that are pivotal for transferring innovative bioprocesses with low technology readiness levels (TRL) to higher levels. State-of-the-art bioprocess development usually starts with e.g. an engineered novel production strain/ cell line that was characterized using standardized small-scale cultivations (e.g. shake flask or microtiter plates) (Wewetzer et al., 2015). Bioprocess development is not only time and cost consuming due to iterative developments but also often experience-based and therefore semi-rational with limited scalability as such (Doran, 1995; Neubauer et al., 2013). In state-of-the-art bioprocess engineering the physiology of the cell within a bioprocess is only measured rudimentary by the key performance indicators (e.g. growth kinetics, titer, yield, volumetric productivity). In fact, the nature of a bioprocess is highly complex involving different levels of "omics" and regulatory/ signaling mechanisms, corresponding to systems biology (Otero and Nielsen, 2010). Therefore, it is tempting to speculate that more and detailed biological insights are required to better understand the interaction between the bioreactor environment and the cellular behavior (Fletcher et al., 2016) in order to design tailored bioprocesses more efficiently for maximal titers, yields and productivities and effective scale-up.

1.2. Advantages and limitations of "omics" technologies

Omics technologies refer to high throughput analyses at all molecular levels in the living cell from genomics over transcriptomics to proteomics and metabolomics (Fig. 1A). Those systemwide biological insights deliver complementary information (Amer and Baidoo, 2021) with genomics demonstrating the fundamental genetic potential, transcriptomics capturing regulation, proteomics reflecting catalyst

* Corresponding author.
E-mail address: nadja.henke@kit.edu (N.A. Henke).

https://doi.org/10.1016/j.biotechadv.2025.108736
Received 20 July 2025; Received in revised form 6 October 2025; Accepted 11 October 2025
Available online 13 October 2025

abundances as well as metabolomics depicting the metabolite abundances and fluxes. Besides the here mentioned major omics levels, the concept of systems biology continuously comes up with new specialized technologies to investigate principles of e.g. epigenetics, actively translated mRNA etc. Systems biology "omics" methods offer promising opportunities for advancing biotechnological research (Wiechert et al., 2022). Bioprocess engineers wish for a better understanding of the impact of bioprocess parameters represented by the bioreactor environment and the complex interactions with the cellular behavior to enable efficient design of tailor-made bioprocesses.

The integration of (multi-)omics approaches can advance bioprocess engineering as it allows a fundamental decoding of bioprocesses from the biological perspective and thus helps identifying and rationalizing suitable bottlenecks. Since cellular bottlenecks can arise across all four omics levels, relying on a single omics technology may not be sufficient for identifying such bottlenecks.

Genomics allows a blueprinting of the complete DNA information of a cell or organism that can be considered more or less static in the context of biotechnological processes. Available genome sequences of industrially relevant microorganisms and cell lines lay the foundation towards the general understanding of metabolic pathways and regulatory repertoires and therefore define the fundamental potential for bioprocesses. Moreover, the genomic information is pivotal for rational strain/cell line design (Grassi et al., 2025) allowing tailored utilization of alternative substrates or the establishment of valuable biosynthetic pathways (Gupta and Lee, 2007). Genomics is recommended for bioprocess investigations when genetic drifts, mutations or copy number variations accumulate in strain/cell lines (Vries et al., 2017; Wurm and Wurm, 2021) e.g. during long-term continuous cultivations.

Transcriptomics in contrast represent a highly dynamic measure that depends on the environmental conditions. Based on its dynamics, transcriptomic analyses in general represent a snapshot of the cellular state that is rapidly changing e.g. across a (fed-) batch process as the environmental conditions, namely nutrient concentrations, are ever changing. Transcriptomics is suitable to identify bottlenecks in the regulation of transcription such as overexpression of negative transcriptional regulators or repression of genes from biosynthetic pathways (Kim et al., 2018).

Proteomics aims to quantify the abundance of proteins/enzymes as well as potential post-translational modifications that are relevant for cellular functions (Yang and Qiao, 2023). In this context, mass spectrometry-based proteomics (Messner et al., 2023) are particularly important if post-transcriptional mechanisms dominate/limit the product biosynthesis in characterized strains/cell lines with the potential to drive metabolic engineering strategies (Yunus and Lee, 2022). Proteomics can enable the identification of insufficient amounts of enzymes or missing post-translational modification that are pivotal for catalytic activity and the overall bioprocess (Gupta and Lee, 2007).

Metabolomics addresses the analyses of all intracellular and/or extracellular metabolites e.g. glycolytic intermediates, lipids, vitamins, cofactors, etc. from investigated bioprocesses (Oldiges et al., 2007). This "omics"- level can be considered as the direct outcome of all biochemical reactions. Diverse analytical platforms can be applied e.g. LC–MS or GC–MS to determine the qualitative and quantitative profiles within bioprocesses. Metabolomics provides the greates insight when bottlenecks arise from cofactor imbalances, precursor deficiencies (Zhou et al., 2023) or the accumulation of toxic/inhibitory byproducts.

More than 20 years ago the field of bioprocess engineering moved slowly from a *know-how* to a *know-why* approach that addresses those detailed and quantitative insights into the cells behavior during a bioprocess (von Stockar et al., 2003). Since then, bridging between bioprocess engineering and omics technologies, primarily metabolomics and fluxomics, offered great opportunities while expanding the mission of bioprocess engineering towards a fundamental understanding of the biological mechanism in a bioprocess (Feng et al., 2010; Wiechert et al., 2007).

Especially, metabolome studies significantly improved the understanding of cellular behavior in bioreactors by providing a comprehensive snapshot of the metabolic state of cells as it allows researchers to monitor hundreds of metabolites (Zhang et al., 2020), identify bottlenecks and detect subtle changes in metabolic pathways that would otherwise go unnoticed (Tanaka et al., 2024). For example, in microbial and mammalian cell cultures, metabolomics has been used to optimize the production of biofuels, therapeutic proteins, and other high-value compounds by pinpointing limiting steps and guiding targeted genetic modifications (Singh et al., 2024). With regard to bioprocess scale-up, a metabolomic profiling of mammalian cell cultures showed comparable intracellular profiles across different scales, thus demonstrating to be a suitable method to gain physiological insights during bioprocess engineering (Vodopivec et al., 2019). In general, both in-line and on-line analytics exist that allow detection of metabolites from the fermentation broth e.g. via different spectroscopic or chromatographic methodologies (Reiter et al., 2021; Rodrigues et al., 2018; Zu et al., 2017). Those approaches usually require mathematical models to identify the different signals in the compound mixture (Marison et al., 2013). Online metabolomics further enhances bioprocess monitoring by enabling high-resolution, real-time data acquisition, which supports better process control and forecasting of key process variables (Cortada-Garcia et al., 2022; Cortada-Garcia et al., 2024).

Metabolome studies are facing two major challenges in the context of

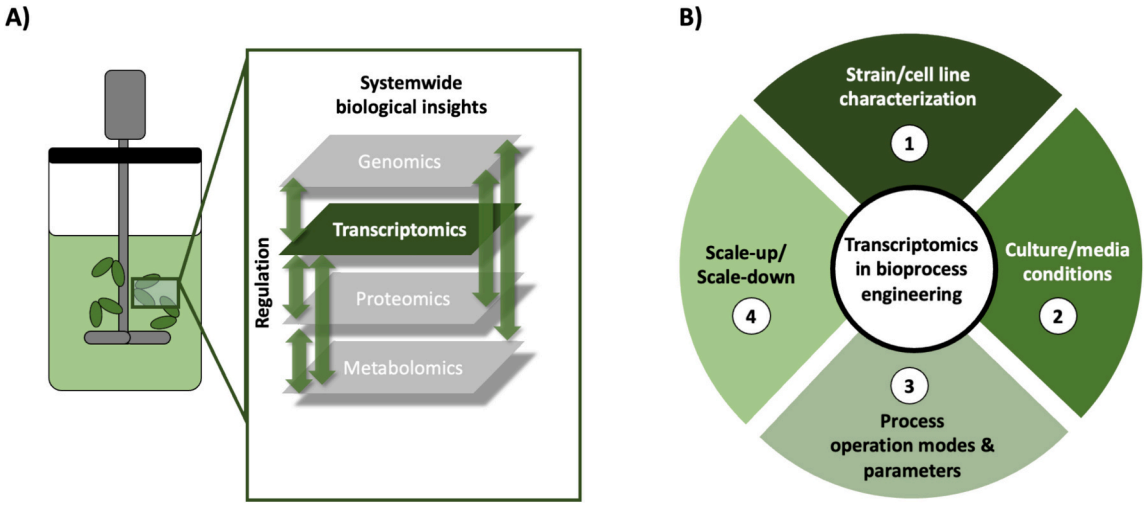

Fig. 1. (A) Omics technologies for application in bioprocessing and (B) application fields of transcriptomics in bioprocess engineering.

holistic biological insights. First, metabolome studies are usually defined by the analytics that are specific regarding qualitative and quantitative measurements of certain metabolites and thus a (small) portion of the entire metabolome can be determined. Few studies applied mass spectrometry for untargeted on-line measurements for the monitoring of more than 800 different *m/z* signals from an *E. coli* fermentation broth (Cortada-Garcia et al., 2022). Secondly, lack of standard calibration curves limits the absolute quantification of identified metabolites.

In summary, metabolomics studies have demonstrated to be a successful methodology to deliver useful biological data to complement bioprocess engineering challenges in the broad context. However, as biological regulation occurs on different levels, transcriptomics harbors the potential to further unravel fundamental gene expression alterations with efficient high throughput screenings such as RNA-seq.

Transcriptional profiling and advanced sequencing technologies (Wang et al., 2009) offer great potential to expand the insights on biotechnological processes following the principle of systems biology (Fig. 1A). Early publications applied transcriptomics more from an observational perspective that have to become more actionable in order to advise industrial productions in terms of an omics-driven biotechnology (Amer and Baidoo, 2021; Masson et al., 2023). Sequencing-based transcriptomics offer some properties that are fundamentally different from metabolomic analysis. Due to the biochemical homogeneity of RNA molecules, especially mRNAs, transcriptomics offers the possibility to track the expression of all genes within any organisms, tissue or cell line. It is reasonable to hypothesize that a better understanding of the biology of a bioprocess will facilitate a shortening of developmental time, a reduction of the risk of up-scaling failure and increased quality (Sieck et al., 2013) of bioprocesses and consequently a reduction of developmental costs.

The scope of this article is to give an overview about the current status of transcriptomics in bioprocess engineering and its potential based on emerging sequencing-based methodologies that allow for spatial and temporal resolution of the transcriptional landscape of bioprocesses. Original research articles applying transcriptomics in bioprocess engineering can be categorized according to the following scopes: (i) strain/cell line physiology characterization, (ii) investigations on culture/media conditions, (iii) process operations as well as (iv) scale-up/down (Fig. 1B).

2. Current status of transcriptomics for bioprocess investigations

High throughput transcriptomic approaches with (c)DNA microarrays were established more than 30 years ago as a hybridization technology that allowed a scalable analysis of gene expression (Hoheisel, 1997; Lockhart et al., 1996; Schena et al., 1995) (Fig. 2). DNA microarrays were widely applied for the characterization of the physiology of various model organisms including microbial systems e.g.

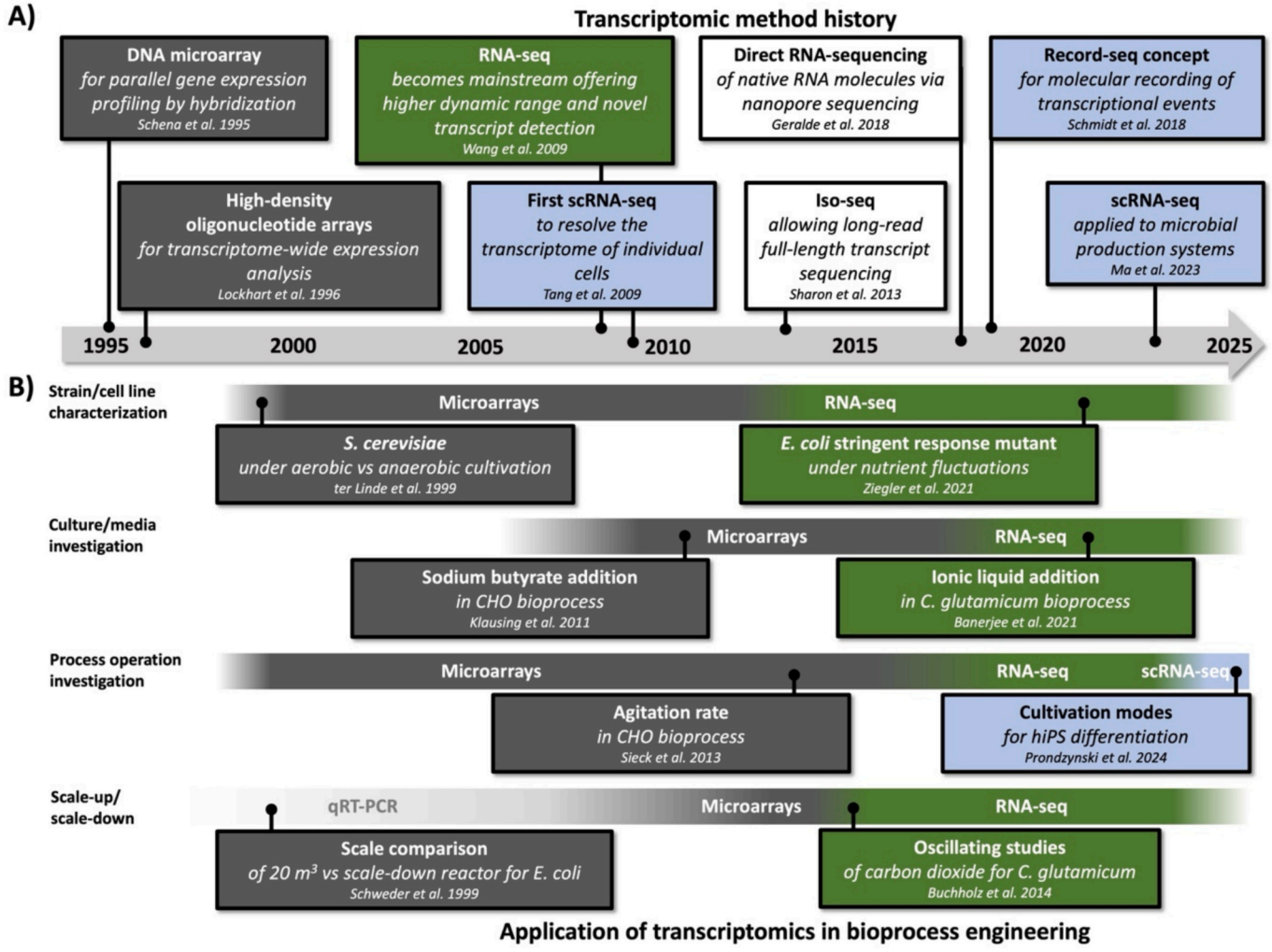

Fig. 2. (A) Timeline of transcriptomics method history and (B) exemplified application in bioprocess engineering based on the four fields (i) strain/cell line physiology characterization, (ii) investigations on culture/media conditions, (iii) process operations as well as (iv) scale-up/down.

Table 1
Characterizing strain/cell line physiology with transcriptomic approaches from the bioreactor environment.

Aim of the study	Set-up and organism	Method	Reference
General strain/cell line characterization	Aerobic and anaerobic chemostat cultivation in stirred 1 L bench-top bioreactors; *S. cerevisiae*	Microarray	(ter Linde et al., 1999)
General strain/cell line characterization	Chemostat cultivation in 800 mL stirred bioreactor; CHO	(miRNA) microarray	(Maccani et al., 2014)
General strain/cell line characterization	Two compartment scale-down bioreactor (3 L stirred tank reactor and 380 mL plug-flow reactor) to examine periodic feast-famine cycles of *E. coli*	RNA-seq	(Löffler et al., 2016)
General strain/cell line characterization	Batch cultivation in bioreactor examining different growth rates; *C. glutamicum*	RNA-seq	(Haas et al., 2019)
General strain/cell line characterization	10 L stirred bioreactor; *Willaertia magna*	RNA-seq	(Hasni et al., 2020)
General strain/cell line characterization	Batch and fed-batch cultivation in 100 mL stirred bioreactor; human PSC	RNA-seq, RT-qPCR	(Rohani et al., 2020)
General strain/cell line characterization	Cultivation in 10 L basket bioreactor; vero cells	qRT-PCR	(Yu et al., 2022)
General strain/cell line characterization	Fed-batch in 15 mL stirred bioreactor to study aggregation of antibodies; CHO	RNA-seq	(Barzadd et al., 2022)
General strain/cell line characterization	Anaerobic 1.7 L stimulus response chemostat cultivation; *S. cerevisiae*	RNA-seq	(Minden et al., 2023)
Comparison of strains	Continuous single and mixed culture fermentation in 1.3 L stirred bioreactor; *S. cerevisiae* and *Lachancea thermotolerans*	RNA-seq	(Shekhawat et al., 2019)
Comparison of strains	Two compartment scale-down bioreactor (3 L stirred tank reactor and 380 mL plug-flow reactor) to examine periodic nutrient depletion of stringent response mutant; *E. coli*	RNA-seq	(Ziegler et al., 2021b)
Comparison of strains	Characterization of an evolved strain in stirred 30 L bioreactor with molasses and glucose; *S. cerevisiae*	RNA-seq	(Wu et al., 2023)
Data delivery for modelling	Chemostat cultivation in 0.75 L stirred bioreactor for improving a genome-scale metabolic model; *P. putida*	Microarray	(van Duuren et al., 2013)

Escherichia coli (Selinger et al., 2000), *Corynebacterium glutamicum* (Wendisch, 2003), *Saccharomyces cerevisiae* (DeRisi et al., 1997), and *Pichia pastoris* (Sauer et al., 2004). In the 2010s, RNA-Seq mainly replaced microarrays due to good quantification properties and easy application for in-depth analysis of regulatory mechanisms (Marguerat and Bähler, 2010; van Vliet, 2010) for various organisms as a reference genome sequence is not necessary for the analysis (Fig. 2A). More than 7200 publications are listed in PubMed under the search criterion "RNA-seq [title]" from 2008 on (Date: July 2025). Among those publications RNA-seq was applied to various cellular systems from microbes to human cell samples for either fundamental research in all kinds of life-science, natural and biomedical research. In microbial biotechnology it was applied e.g. for analysis of regulatory networks (Dostálová et al., 2018) or general stress responses (Küberl et al., 2020) as well as for guiding of metabolic engineering of (in)direct pathways (Zhao et al., 2022) or for the analysis of laboratory evolved production strains (Wang et al., 2018).

In comparison to molecular biology/biomedicine, where the above-mentioned methods are established and state-of-the-art since 2010, application of those methodologies in the context of bioprocess engineering became more and more important over the last years (Fig. 2B).

2.1. Investigating strain/cell line characteristics

Transcriptomics was applied for general characterization of strains/cell lines as well as for comparison of strains/cell lines or for delivery of holistic data (Table 1). Already 25 years ago the group of Pronk studied global transcriptional adaptation to aerobic and anaerobic cultivation conditions in *S. cerevisiae* from chemostat cultivations (ter Linde et al., 1999). It was demonstrated that more than 350 genes were upregulated either under aerobic or anaerobic conditions indicating the broad biological alterations in a bioprocess. A recent study by Minden et al. performed stimulus response chemostat cultivations, mimicking mixing-induced heterogeneities of large bioreactors via glucose limitation under ethanol production of *S. cerevisiae* (Minden et al., 2023). In more detail, it was demonstrated that a single limitation–-starvation–limitation transition showed a differential gene expression with more than 1000 genes that peaked about 10–20 min after the stress and relaxed at about 60 min post glucose depletion.

Further transcriptional analyses of the industrial cell factory *C. glutamicum* were applied to characterize different growth rate (transitions) in a batch bioprocess. In combination with metabolomics, it was possible to identify genes associated with growth, namely the growth modulon, describing a functional group of genes associated with the growth rate (Haas et al., 2019). The same growth rates (0.2, 0.3, 0.4 h^{-1}) were transcriptionally analyzed under glucose limited chemostat conditions (Graf et al., 2020) highlighting that 547 genes, corresponding to approximately 20 % of the annotated genes, were differentially expressed across the examined flow rates. Both studies indicate the relevance of further deep and systematic transcriptional analysis for a full picture of biological responses across diverse process types.

RNA-seq could also be applied to the amoeba *Willaertia magna* in 10 L bioreactors in order to understand its metabolism and molecular mechanisms under axenic mass culture (Hasni et al., 2020). This study showed that this amoeba has the ability to adapt to low oxygen concentrations and moreover investigated the transcripts associated with its role as a cellular predator in its natural habitat.

Research by Ziegler et al. and Wu et al. applied RNA-seq to explain performance benefits of a stringent response mutant of *E. coli* and of an evolved strain of *S. cerevisiae* grown on molasses, respectively (Wu et al., 2023; Ziegler et al., 2021b). The stringent response mutant of *E. coli* outperformed the parental strain in terms of the eGFP content by approximately 50 % based on a reduced maintenance that was achieved by deletion of genes that were highly overexpressed under repeated transient starvation (Löffler et al., 2016).

Transcriptomics is most useful in strain and cell line development when it reveals bottlenecks that are directly controlled at the transcriptional level mediated by transcriptional regulators or when up/down regulation of genes directly improves the productivity (Wu et al., 2023; Ziegler et al., 2021b). However, many studies detect quite a great number of differentially expressed genes without clear molecular causality reflecting that the behavior might be explained by protein stability and/or metabolic fluxes. Therefore, transcriptomics is best suited to identify transcriptional regulator (networks) as rational impetus for metabolic engineering or adaptive laboratory evolution, whereas proteomics and fluxomics are better suited to identifying catalytic and post-translational limitations. Transcriptomic data from bioprocess allow high throughput hypothesis generation as basis for subsequent strain

improvement (Hasni et al., 2020; Minden et al., 2023).

2.2. Investigating culture/media conditions

The effect of culture and media conditions was analyzed across diverse cellular systems from microbial to mammalian monocultures with transcriptomics approaches (Table 2). RNA-seq was used to investigate the effect of different or mixed substrates for various microbial bioprocesses. For *Pichia pastoris* RNA-seq analysis from a protein production process demonstrated that a glycerol-methanol feeding phase prior to the methanol feeding phase helps to reduce the negative effects from methanol utilization (Zhang et al., 2020). It was found that such intermediate feeding phase facilitates adaptation of the cells from glycerol to methanol utilization mainly by upregulation of the MAPK-pathway genes resulting in an improved cell wall integrity with benefits under high methanol presence. Another study analyzed the differentially expressed genes in a *S. cerevisiae* bioprocess producing ribonucleic acids when glucose or molasse were used as carbon source (Wu et al., 2023). Interestingly, glucose utilization resulted in an upregulation of genes associated with biosynthesis of sulfur-containing amino acids (methionine, cysteine, adenosylmethionine) as well as genes from the assimilatory sulfate reduction pathways, thus contributing to an improved RNA biosynthesis and methylation that was in accordance with the process performance. Quantitative transcriptomics could indeed unravel that the iron chelator protocatechuic acid (PCA) from the CGXII minimal medium served as a hidden co-substrate for accelerated growth of the industrial workhorse *C. glutamicum* (Unthan et al., 2014) as genes encoding for the enzymes responsible for the PCA uptake and metabolism of PCA were significantly upregulated in the first growth phase.

The widely used treatment of Chinese Hamster Ovary (CHO) bioprocesses with sodium butyrate was investigated with microarray and later RNA-seq. Among others the research group of Noll analyzed the underlying mechanisms of sodium butyrate addition for improved antibody production (Klausing et al., 2011). The cDNA-based microarray analysis revealed that addition of sodium butyrate in a batch process resulted in a downregulation of genes associated with cell growth as well as an upregulation of genes associated with carbon and amino acid biosynthesis that corresponds to an reduced cell proliferation and an increased antibody titers (Klausing et al., 2011). Another study applied the DNA microarray methodology in chemostat cultivations in spinner flasks explaining that addition of sodium butyrate as well as low temperatures possess different but synergistic gene expression profiles that facilitate resting cells in G0/G1 phase (Avello et al., 2022). 10 years later, a similar study revealed the mode of action of butyric acid in intensified fed-batch bioprocesses in stirred bioreactors with RNA-seq (Schulze et al., 2022).

For investigations of media components, transcriptomics can help identifying global cellular reprogramming e.g. in favor for antibody production as shown for CHO cells (Avello et al., 2022; Klausing et al., 2011) or can explain the positive effect of substrates and additional media components in bioprocess development (Banerjee et al., 2021; Wang et al., 2024). The work from (Unthan et al., 2014) demonstrated that even subtle nutrient shifts can result in obvious differentially expressed genes allowing different growth rates. As not all gene expression alterations alone could be directly linked to the cell's physiology, integration of proteomics and metabolomics can help in linking gene expression changes to e.g. anabolic/catabolic reactions and nutrient fluxes. Therefore, nutrient dependencies in media optimization can be revealed at the transcriptional level, while fine-tuning might need to be complemented by metabolomic analytics.

2.3. Investigating process operation modes and parameters

Several studies deal with the investigation of process operations based on transcriptomics approaches (Table 3). As an example, the impact of the agitation rate/stirrer speed was investigated as relevant process parameter in eukaryotic bioprocess operations. Sieck et al. analyzed the impact of hydrodynamic stresses that occur in industrial CHO processes based on stirring and sparging (Sieck et al., 2013). Interestingly, only 57 genes were differentially expressed under hydrodynamic stress, whereas most of them were associated with DNA damage and repair mechanisms. However, there was no direct explanation for the reduced antibody titers measured under hydrodynamic conditions based on the microarray data.

Moreover, in cell culture bioprocess engineering comparative transcriptomics (RNA-seq, microarray and qRT-PCR) investigated similarities and differences between 2D and 3D cultivation systems (Fenouil et al., 2023; Yu et al., 2022) as well as stirred scale-down bioreactors examining shear stress (Gareau et al., 2014).

Another study examined the dynamic interplay between the oxygen availability, growth rates and the transcriptional response of the microbe *Yarrowia lipolytica* (Kerssemakers et al., 2023). Large scale bioreactor conditions were simulated as a compartmental modelling approach and the organism's behavior in these relevant surroundings. With the help of a design-build-test-learn cycle considering substrate,

Table 2
Investigating culture/media conditions with transcriptomic approaches.

Aim of the study	Set-up and organism	Method	Reference
Investigating carbon sources (carbon dioxide)	Submerged batch cultivation in 5 L stirred bioreactor examining carbon limitation; *Aspergillus niger*	Microarray	(Nitsche et al., 2012)
Investigating carbon sources (protocatechuic acid and glucose)	Batch cultivation in 1.5 L stirred bioreactor examining growth rates and addition of PCA presence; *C. glutamicum*	Microarray	(Unthan et al., 2014)
Investigating carbon sources (glycerol and methanol)	High cell density fed-batch cultivation in 10,000 L; *P. pastoris*	RNA-seq	(Zhang et al., 2020)
Investigating carbon sources (malate)	Fed-batch cultivation in 15 L stirred bioreactor; *Schizochytrium*	RNA-seq	(Zhang et al., 2022)
Investigating carbon sources (glycerol and glycerol/methanol mixtures)	Fed-batch cultivation in 2 L stirred bioreactor feeding; *P. pastoris*	RNA-seq	(Boojari et al., 2023)
Investigating carbon sources (molasses and glucose)	Characterization of an evolved strain in stirred 30 L bioreactor; *S. cerevisiae*	RNA-seq	(Wu et al., 2023)
Investigating carbon sources (xylose and shrimp shell waste)	Utilization of xylose and shrimp shell waste in 5 L bioreactor; *Clostridium tyrobutyricum*	RNA-seq	(Wang et al., 2024)
Investigating medium additive (sodium butyrate)	Stirred 2 L bioreactor batch process; CHO	Microarray	(Klausing et al., 2011)
Investigating medium additive (sodium butyrate)	Chemostat cultivation in 250 mL spinner flasks; CHO	Microarray	(Avello et al., 2022)
Investigating medium additive (sodium butyrate)	Fed-batch in 5 L stirred bioreactor with butyric acid supplementation; CHO	RNA-seq	(Schulze et al., 2022)
Investigating growth rates	Chemostat cultivation in bioreactor; *C. glutamicum*	RNA-seq	(Graf et al., 2020)
Investigating ionic liquid stress	Fed-batch cultivation in 2 L stirred bioreactor; *C. glutamicum*	RNA-seq	(Banerjee et al., 2021)

Table 3
Investigating process operation modes and parameters with transcriptomic approaches.

Aim of the study	Set-up and organism	Method	Reference
Impact of process parameter (agitation rate)	Stirred 2 L bioreactor examining hydrodynamic stress based on agitation rate; CHO	Microarray	(Sieck et al., 2013)
Impact of process parameter (agitation rate)	Stirred bioreactor to investigate shear stress based on agitation rate; pluripotent embryonic stem cells	Microarray/ qRT-PCR	(Gareau et al., 2014)
Impact of process parameter (oxygen)	3 L stirred bioreactor investigating anaerobic to aerobic transitions; *E. coli*	RNA-seq	(Bui and Selvarajoo, 2020)
Impact of process parameter (temperature)	Chemostat cultivation in 250 mL spinner flasks examining temperature and sodium butyrate; CHO	Microarray	(Avello et al., 2022)
Impact of process parameter (dilution rate and oxygen)	Chemostat cultivation in bioreactor examining different dilution rates and dissolves oxygen; *Yarrowia lipolytica*	RNA-seq	(Kerssemakers et al., 2023)
Bioreactor type	Cultivation in 10 L basket bioreactor; vero cells	qRT-PCR	(Yu et al., 2022)
Bioreactor type	Pressurized batch cultures in hungate tubes and schott bottles; *Pseudothermotoga elfii (*subsp. *lettingae)*	RNA-seq	(Fenouil et al., 2023)
Bioprocess mode	Aerobic and anaerobic chemostat cultivation in stirred 1 L bench-top bioreactors; *S. cerevisiae*	Microarray	(ter Linde et al., 1999)
Bioprocess mode	Batch and fed-batch cultivation in 100 mL stirred bioreactor; human PSC	RNA-seq, qRT-PCR	(Rohani et al., 2020)
Bioprocess mode	Investigating biological batch effects in bioreactor-differentiated hiPSC-CMs (stirred 100 mL bioreactor) and monolayer-differentiated hiPSC-CMs monolayer; human iPSC-derived cardiomocytes	scRNA-seq	(Prondzynski et al., 2024)

oxygen and growth rate heterogeneities it could be shown that the relation between oxygen availability and differential gene expression was dynamic.

Transcriptomic profiling under varying process parameters such as aeration allows actionable insights when the perturbation directly engages transcriptional alterations (Bui and Selvarajoo, 2020; Kerssemakers et al., 2023) that could be addressed in future bioprocess development studies. However, in specific cases such as high shear stress, differential gene expression does not allow for straight forward identification of causative genes even when product titers collapse (Sieck et al., 2013). Then, combining transcriptomic readouts with e.g. process analytical technology (PAT) data or proteomics provides a more reliable basis for process optimization. Thus, the suitability of transcriptomics for process improvement strongly depends on whether the examined parameter disturbance has a transcriptional footprint that is the current bottleneck of the bioprocess.

2.4. Investigating scale-up/down

The understanding of industrial bioprocesses was fostered by transcriptomic methodologies (Table 4). For scale-up/down investigations, already more than 25 years ago transcriptional analyses were applied for the understanding of cellular behavior in bioreactors of different scales. In 1999, Schweder et al. had a close look on the gene expression levels of four stress (*clpB*, *dnaK*, *uspA*, and *proU*) and three oxygen/glucose (*pfl, frd,* and *ackA*) related genes with qRT-PCR from an industrial 20,000 L and a scale-down bioreactor with *E. coli* (Schweder et al., 1999). It turned out that *ackA* and *proU* were highly expressed in the batch phase of the scale-down bioreactor and decreased during feeding phase, whereas the other stress genes stayed relatively constant across the process. In contrast, in the industrial bioreactor most of the stress related genes were upregulated in the middle and top part of the bioreactor when glucose was fed. Thus, demonstrating that the behavior of microorganisms in large-scale bioprocesses is different from bench-top bioreactors. Those findings are in accordance with the widely known existence of chemical and physical gradients (e.g. nutrients, solved gases) in industrial settings highlighted e.g. with CFD simulations (Blöbaum et al., 2023; Lara et al., 2006a, 2006b).

Further studies harnessing microarray and RNA-seq compared the transcriptional status of eukaryotic bioprocesses across different scales. The Martens group demonstrated that a well-defined scale-down of a CHO fed-batch process from 10 L to 15 mL system aided by tip seed or power input indeed results in less than 6 % of gene expression variation (Alsayyari et al., 2018). For mammalian cell culture systems it was

Table 4
Scale-up/down investigations applying transcriptomics approaches.

Aim of the study	Set-up and organism	Transcriptomic method	Reference
Scale comparison	Industrial stirred bioreactor tank with 20,000 L and scale-down reactor; *E. coli*	qRT-PCR	(Schweder et al., 1999)
Scale comparison	Scale-down experiments from 5 L to 35 mL stirred bioreactor; Sp2/0 cells	Microarray/ qRT-PCR	(Kondragunta et al., 2012)
Scale comparison	Stirred bioreactor systems of two scales; Baby hamster kidney	Microarray	(Jayapal and Goudar, 2014)
Scale comparison	Scale-down experiments from 10 L to 15 mL stirred bioreactor; CHO	RNA-seq	(Alsayyari et al., 2018)
Scale-down (oscillating oxygen)	Two-compartment scale-down reactor; *E. coli*	qRT-PCR	(Lara et al., 2006a, 2006b)
Scale-down (oscillating carbon dioxide)	A three-compartment cascade bioreactor; *C. glutamicum*	RNA-seq	(Buchholz et al., 2014)
Scale-down (oscillating oxygen and substrate)	Two-compartment scale-down reactor; *C. glutamicum*	Microarray	(Käß et al., 2014)
Scale-down (oscillating ammonia and glucose)	Two compartment scale-down reactor (3 L stirred tank reactor and 380 mL plug-flow reactor); *E. coli*	RNA-seq	(Simen et al., 2017)
Scale-down (oscillating nitrogen)	Two compartment scale-down reactor (3 L stirred tank reactor and 380 mL plug-flow reactor) to examine periodic nutrient depletion of stringent response mutant; *E. coli*	RNA-seq	(Ziegler et al., 2021b)

hypothesized that gene expression data could be a substantially stronger indications for similarity between production processes (Jayapal and Goudar, 2014) in comparison to other key performance indicators.

Moreover, transcriptional insights were used to unravel e.g. the influence of oxygen, carbon dioxide or substrate oscillations during scale-down studies. Systematic investigations, based on RNA-seq and microarray, were performed with *C. glutamicum* examining the process parameters CO_2 (Buchholz et al., 2014) as well as O_2 and glucose (Käß et al., 2014). Buchholz and colleagues found that fluctuations in CO_2 resulted in approximately 66 differentially expressed genes after 3.6 min of stimuli presence but process performance stayed unaffected with the examined lysine producing strains (Buchholz et al., 2014). Accordingly, Käß and colleagues showed that repeated O_2 and glucose fluctuations had no impact on the process performance of the same DM 1933 lysine producing strain indicating the strains robustness (Käß et al., 2014).

The effect of glucose and ammonia oscillations were analyzed with a two-compartment bioreactor and RNA-seq studies of *E. coli* (Simen et al., 2017; Ziegler et al., 2021b). The characterization of nutrient fluctuation in such settings aims on the understanding of the metabolic control that occur when microbes flow through the different zones of a large tank that is also known from the concept of microbial lifelines (Blöbaum et al., 2023). In general, mixing inhomogeneities increase with increasing bioreactor dimensions and thus interfere with the direct conclusion of bioprocess behavior from lab to industrial scale. Takors and colleagues studied the differentially expressed genes in a continuous two compartment bioreactor system comprising a STR (ammonia limitation zone) and a plug-flow reactor (ammonia starvation zone). In this scale-down setting it was found that about 400 genes were repeatedly turned on and off in *E. coli* indicating an increased maintenance demand by 15 % (Simen et al., 2017). These insights were subsequentially used to construct a deletion mutant of *E. coli* with a reduced maintenance coefficient that has been proven to be beneficial under repeated starvation stimuli for protein production (Ziegler et al., 2021a). Moreover, these findings were in accordance with the characterization of a stringent response deficient mutant of *E. coli* as it was demonstrated that the ppGpp-deficient strain showed substantially reduced short-term transcriptional responses with remaining function of ammonium uptake and biomass formation (Ziegler et al., 2021b).

Application of transcriptomics in scale-down experiments can provide actionable insights, particularly when large-scale heterogeneities (e.g. substrate or oxygen gradients) correspond to significant transcriptional responses as e.g. in the case of the stringent response or shifts in energy metabolism (Löffler et al., 2016; Simen et al., 2017). Such insides have proven to rationalize the engineering of strains with superior behavior under mimicked large scale process conditions (Ziegler et al., 2021a; Ziegler et al., 2021b). In some cases, large-scale phenomena (e.g. CO_2 stripping or mechanical mixing) result in transcriptional data that only provide correlative context without clear causality. Therefore, transcriptomics is a powerful tool for diagnosing scale-dependent stresses, but process improvement might require complementary omics to fully understand the cellular behavior.

2.5. From transcriptomics insights to improved strains and bioprocess designs

Transcriptomic insights have been demonstrated to be translatable into improvements of the examined bioprocesses as represented by some of the studies described in Section 2. At the intersection between strain characterization and scale-down, RNA-seq analysis revealed that *E. coli* cells confronted with oscillating environments in scale-down experiments are frequently transcriptionally reprogrammed e.g. in terms of the stringent and general stress response. Those data successfully guided the design of a robust *E. coli* strain that showed about 50 % higher eGFP yield in mimicked large-scale bioprocesses (Löffler et al., 2016; Ziegler et al., 2021a). Transcriptomic analyses in *P. pastoris* have shown that carbon source-specific responses occur primarily on the transcriptional level (Prielhofer et al., 2015). This insight has enabled the design of improved bioprocess strategies, including the implementation of an additional glycerol plus methanol feeding phase prior to the methanol induction (Zhang et al., 2020). Zhang and colleagues identified that the upregulation of genes associated with MAPK signaling pathways as well as of the autophagy mechanism could rationalize the improved biomass titer by approximately 20 % and an improved phythase activity as measure of the protein production by approximately 50 % (Zhang et al., 2020). Moreover, transcriptomic analyses facilitated the rationalization of bioprocess characteristics across all four application scenarios described in Section 2, representing an important first step towards the understanding and subsequent optimization of those processes. With continued advances in the field, such insights may ultimately be translated into improved bioprocesses.

3. Sequencing-based transcriptomics for bioprocesses: advantages and constraints

3.1. RNA-seq benefits and workflow principles

Transcriptomics methods like RNA-seq or direct RNA sequencing represent commonly used methods to systematically characterize gene expression in various cell systems and organisms. The application of transcriptomics, both in fundamental research and in strain/cell line development, is based on several considerations.

1.) Gene expression is an important basis for the adaptation of a cell to its environment thus quickly responding to any changes of environmental conditions detected by the organism. Changes in gene expression often take place before the cell enters a detrimental physiological route that could e.g. result in cell death. Thus, a continuously changing adaptation of gene expression is essential for cell survival.
2.) RNA-seq enables a systematic and efficient analysis of all transcript abundances in a cell or organism at a defined time point under defined conditions – the transcriptome. The homogeneous nature of all RNA molecules is crucial for this accessibility. While mRNAs derived from different genes vary substantially with respect to the order of ribonucleotides, these differences are neglectable with respect to the overall biochemical properties of the macromolecule (Shilpha et al., 2024; Wang et al., 2009).
3.) RNA-seq allows a simultaneous investigation of RNAs derived from protein-encoding and regulatory genes. Direct RNA sequencing reveals modification of the RNA that might have a functional consequence. Insights into the regulation of genes can be inferred from the resulting transcriptomic data sets (Liu-Wei et al., 2024).
4.) A high sensitivity and a large dynamic range of sequencing-based transcriptomics enables the simultaneous quantification of gene expression across orders of magnitude >8000 fold (Wang et al., 2009). The high throughput analysis enables an affordable prize for RNA-seq experiments with a large number of samples.
5.) Emerging transcriptomics applications (Table 5) like single-cell RNA-seq lead to higher temporal or spatial resolution of data sets. Comparison at the single cell level is feasible today (Heumos et al., 2023).

In the following, state-of-the-art workflows of sequencing-based transcriptomics are illustrated (Fig. 3). These workflows are largely similar but differ in specific steps. Cells of interest are separated from culture media. Mechanical force like incubation in a ribolyser is used to disrupt the cell envelope which releases the mRNA containing cytoplasm. This step can be replaced by enzymatic degradation of cell enclosing structures if applicable. Various methods exist for the extraction of RNA from the mixture of cell components. Frequently deployed are dedicated kits that separate RNA from other cell

Table 5
Selected types of sequencing-based transcriptomics and outdated technologies for comparison.

Transcriptomics technologies and principle	Advantages and limitations	Application scenarios and costs	Reference
Microarrays Hybridization to probe	+ First high-throughput method for transcriptomics - Only quantification of represented molecules; small dynamic range	Gene expression comparison between two samples; very high setup costs	(Bumgarner, 2013)
mRNA-Seq Sequencing by synthesis (Illumina)	+ Systematic quantification of transcript abundances - No information about small regulatory RNAs	Gene expression quantification; low costs	(Wang et al., 2009)
direct RNA sequencing Oxford Nanopore Technologies (ONT)	+ Identification of RNA modifications - Very high RNA input required	RNA modification analysis; high costs	(Garalde et al., 2018)
Iso-Seq(PacBio)	+ Full length cDNA sequencing - High RNA input required	Full length transcript analysis; high costs	(Sharon et al., 2013)
Emerging technologies	**Advantages and limitations**	**Application scenarios and costs**	**Reference**
scRNA-Seq Sequencing by synthesis (Illumina)	+ Single cell level resolution - Only highly expressed genes captured	Revealing diversity of cell population; very high costs	(Tang et al., 2009) (Tang et al., 2010) (Tang et al., 2011)
Live-Seq Sequencing by synthesis (Illumina)	+ Repeated sampling and sequencing from single cell - Technically challenging	Monitoring of transcriptome of single cell; high costs	(Chen et al., 2022)
Record-Seq Sequencing by synthesis (Illumina)	+ Information about succession of gene expression - Customization for application required	Temporally resolved analysis of bioprocess; high costs	(Schmidt et al., 2018) (Tanna et al., 2020)
Ultima Genomics	+ Systematic quantification at very low costs - Low read accuracy	Gene expression quantification; very low costs	(Almogy et al., 2022)
SBX Roche	+ Single molecule sequencing with high accuracy - Unknown	Full length transcript analysis; unknown	(Kokoris et al., 2025)

compounds through selective binding to a silica membrane in a filter column. Due to similar biochemical properties, DNA is often co-purified at this step. A treatment with RNase-free DNase can help to remove any residual DNA. Depletion of rRNAs can be performed based on magnetic beads that carry complementary probes on their surface. rRNAs are bound to the probes on the magnetic beads and removed by pulling down all beads with a magnet. mRNA can be reverse transcribed into cDNA for RNA-seq (Illumina, BGI-Seq), full length cDNA sequencing (ONT), or Iso-Seq (PacBio) (Table 5). It is also possible to sequence RNA directly (ONT) by constructing a complementary cDNA strand for the initiation of the sequencing reaction. While modern long read sequencing technologies provide the opportunity to obtain full length cDNA sequences, RNA-seq relies on cDNA fragments of a certain size. Sheared cDNA is size selected and subjected to the construction of a library based on the Illumina/BGI protocol. Simultaneous sequencing of multiple samples on the same device is possible due to the integration of barcodes during the library preparation stage. Reads are assigned to individual samples based on their barcodes after completion of the sequencing experiment (Illumina, PacBio) or during the sequencing experiment (ONT). Details about the sequencing technologies have been reviewed elsewhere (Metzker, 2010; Pucker et al., 2022; Pucker and Schilbert, 2019).

3.2. Technical challenges and limitations of transcriptomics in bioprocess engineering

While transcriptomics has the potential to inform bioprocess engineering, there are several technical and economic constraints that might limit its practical application. Extraction of RNA from bioreactor samples requires protocol optimization to ensure high and consistent RNA quality. State-of-the-art autosamplers next to bioreactors in conjugation to the usage of DNA/RNA Shield™, RNA*later*® or other preserving agents allow proper storage of culture samples for transcriptomics protocols (Menke et al., 2017). This automatic and optimized sampling with subsequent RNA extraction would be feasible for routine applications which are required for bioprocess application. However, a cost-benefit analysis would be especially important for routine process monitoring. As gene expression is highly dynamic snapshots from single time points may not capture transient or oscillatory responses important for process monitoring/optimization. Therefore, a dense sampling scheme in the minute-to-hour range would be required to achieve a sufficient temporal resolution to monitor the transcriptional changes across the whole bioprocess or alternatively at specific critical periods in the bioprocess. Such large-scale studies make transcriptomic analyses expensive and logistically challenging, however it is technically possible. In addition, such high-dimensional transcriptomic datasets require advanced bioinformatic and system biology expertise in order to distinguish meaningful biological signals from noise.

A frequent criticism against transcriptomics is that it is utilized as a proxy for proteomics and metabolic flux analyses. Transcript abundances do not always correlate with protein levels and/or enzymatic activity, limiting direct translation to process performance predictions. Possible translational and post-translational regulation might lower the correlation between the presence of a transcript and the activity of the corresponding enzyme. Due to transcriptional 'bursts', the average correlation can be 0.35–0.45 for individual eukaryotic cells (Buccitelli and Selbach, 2020; Fulcher et al., 2024). However, studies conducted on bulk analyses in microbial systems reported substantially higher correlation values above 0.5 and up to 0.89 (Greenbaum et al., 2003; Houser et al., 2015; Maier et al., 2009). The possibility to analyze a transcriptome at substantially lower costs and with higher throughput in comparison to proteomics makes it an attractive alternative – even if the ultimate understanding of biological behavior might be biased in terms of the ultimate outcome of transcriptional alterations on the other omics level. The integration of metabolomic data can help to validate metabolic flux predictions inferred from RNA-seq data. Translating transcriptomic insights into actionable bioprocess strategies may requires complementory proteomics, metabolomics, and fluxomics data for a full picture.

4. Emerging sequencing-based transcriptomics as enablers of future bioprocess engineering

In the following two advanced RNA-seq methodologies are represented that could guide a way towards understanding the diversity of populations in time and space as an expansion of the toolbox for

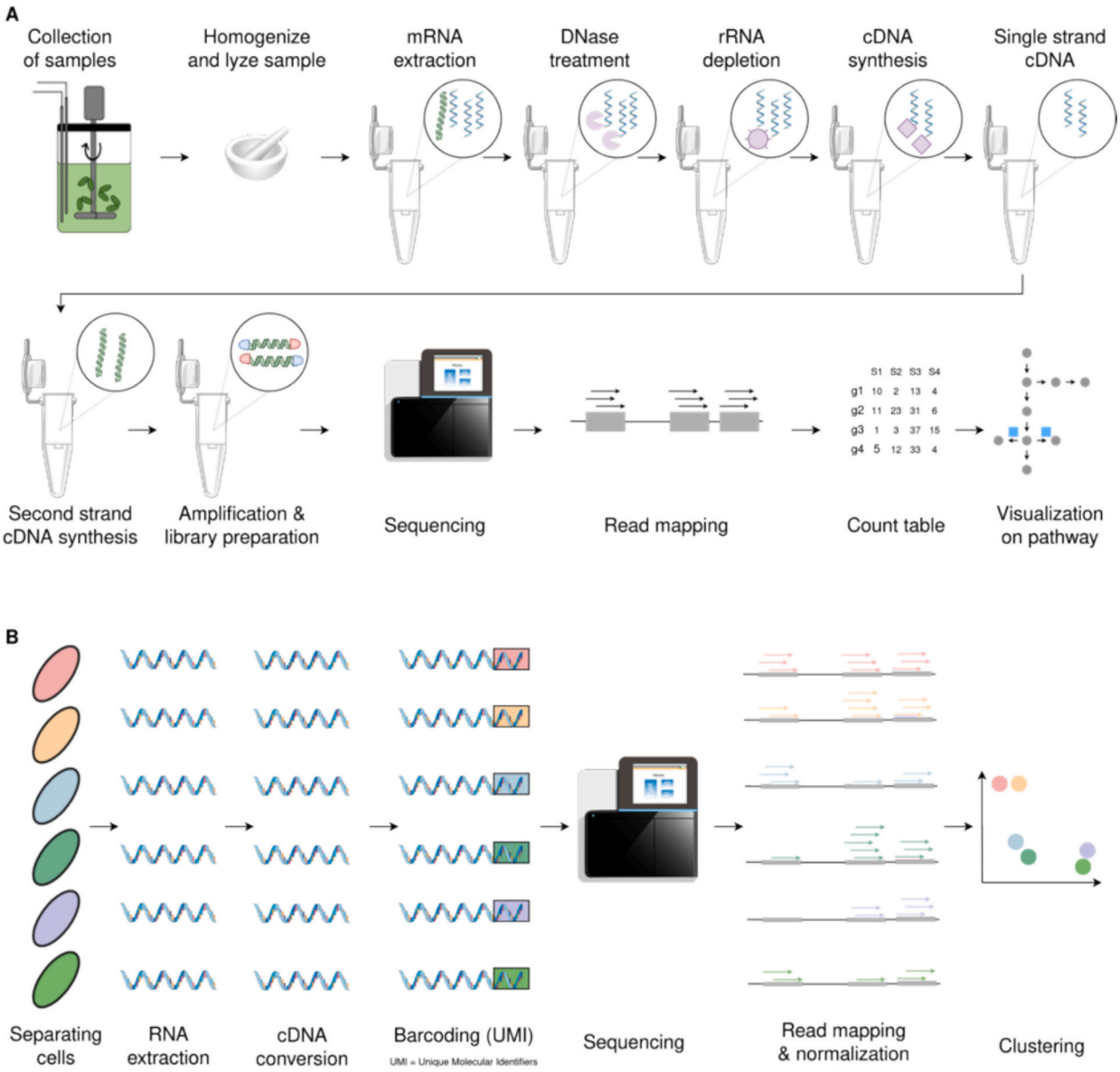

Fig. 3. Simplified workflows of sequencing-based transcriptomics for bioprocesses. (A) A classical mRNA-seq workflow, (B) single-cell RNA-seq.

transcriptomics application in bioprocess engineering in order to unravel so far hidden biological mechanisms/bottlenecks (Fig. 4).

4.1. *Single cell RNA-seq for populational insights*

In comparison to state-of-the-art bulk RNA-seq, that represents an averaged measure across a cell population, single cell RNA-seq (scRNA-seq) allows to determine transcriptional profiles of single cells. Cell-to-cell heterogeneity is an intrinsic property of microbial and mammalian populations in bioreactors, arising from stochastic gene expression, metabolic variability and dynamic environmental conditions (Delvigne and Goffin, 2014). This phenotypic diversification can strongly influence process performance, as subpopulations with distinct growth, productivity, or stress-response profiles determine overall yield, robustness, and scalability (Xu et al., 2024). Therefore, understanding and controlling heterogeneity represents both a fundamental scientific challenge and a key opportunity to design next-generation, highly efficient bioprocesses (Delvigne and Goffin, 2014).

Applying single-cell RNA-seq to bioreactor studies allows researchers to link molecular phenotypes to functional traits such as growth, productivity or stress responses under dynamic culture conditions and thus unrevealling existing subpopulations in bioprocesses. By integrating scRNA-seq data with process parameters, it could be envisioned to map regulatory networks at the single-cell level and uncover drivers of population diversification. Recent work demonstrated that unsupervised scRNA-seq clustering of mCM and bCM cultures could identify the dramatically lower population fraction of non-cardiomyocytes derived from the bioreactor differentiation (Prondzynski et al., 2024). Moreover, scRNA-seq has potential to study adaptation in chemostats at multiple regulatory levels, as adaptation affects the genome, transcriptome, metabolome, and proteome (Wright et al., 2020). Researchers envision to apply single-cell omics technologies, including scRNA-seq, for the holistic study of biological adaptive mechanisms (Baysoy et al., 2023; De Jonghe et al., 2024; Inayatullah et al., 2025).

Technically, scRNA-seq from bacteria showed to be more challenging as cells are harder to lyse, possess a low RNA content in the femtogram magnitude, low mRNA copy number, and transcripts lack functional poly(A) tailing compared to e.g. mammalian cells (Homberger et al., 2022). Recent methodological developments tackled those issues and allow bacterial scRNA-seq (Ma et al., 2023). The BacDrop methods

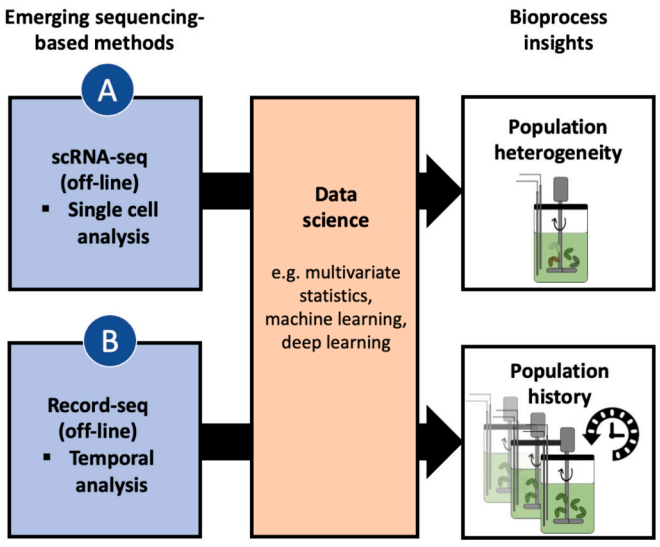

Fig. 4. Emerging sequencing-based transcriptomics technologies and data sciences for envisioning populational (A) and temporal (B) transcriptional profiling of bioprocesses.

includes a rRNA depletion and DNA removal step in the fixed and permeabilized cells with RNase H and DNase I, respectively. Afterwards, unique molecular identifiers (UMIs) are added to the 5′ end of the cDNA ("plate barcode") via reverse transcription in 96-/386-well plates. Cells are pooled and cDNA is polyadenylated at the 3′ end via terminal transferase. Then droplets are generated, and a second ground of barcoding is conducted ("droplet barcode") as well as the second strand cDNA synthesis with oligo-dT priming. The fully captured cDNA contains UMIs, plate and droplet barcode for minimal cell barcode collision rates of less than 10 % (Ma et al., 2023). Finally, cDNA is amplified, and library is constructed for Illumina sequencing. Besides the microdroplet-based method, other cell isolation methods exist, namely, laser capture microdissection (LCM) and capillary pipettes guided by a microscope from solid samples as well as fluorescent-activated cell sorting (FACS) enables cell isolation from liquid samples (Moon et al., 2023). Single cell RNA-seq was already demonstrated for a wide range of bacteria species (Homberger et al., 2022) and indicated to be a powerful tool to characterize population dynamics in high throughput.

The key advantage of scRNA-seq lies in its ability to resolve transcriptional heterogeneity, revealing subpopulations that are invisible to bulk analyses. This provides deeper mechanistic insight into stress responses, metabolic specialization, and regulatory diversity within bioreactors. However, the method has current limitations: protocols are optimized for mammalian cells rather than microbial cells making lysis and RNA capture challenging (Homberger et al., 2022), data sets are large, noisy and sparse requiring advanced computational analyses and interpretation (Kulkarni et al., 2019) and high costs and laborious sample preparation workflows with limited throughput constrain its routine application. Thus, while scRNA-seq provides unprecedented resolution for understanding population heterogeneity, significant methodological and translational hurdles must still be overcome before it can be fully integrated into mainstream bioprocess development.

Though scRNA-seq application is still relatively limited in bioprocess engineering compared to other fields, the rapid advancements the technology may make it an important supplement to other techniques like flow cytometry (Heins et al., 2022) and microfluidic cultivation systems for studying bioprocesses (Grünberger et al., 2014). In this combination, scRNA-seq insights into cell-to-cell heterogeneity at various environmental conditions can be collected, which is currently not feasibly and/or restricted to fluorescence-based assays only enabling the investigation of single/dual features of the cells. While single-cell RNA-seq would provide higher resolution regarding the diversity of cells in a culture, bulk analyses might be a more economic choice in routine applications.

4.2. *Record-seq for cellular autobiographies*

Unlike endpoint omics methods (see Introduction), which provide only snapshots of population states, Record-seq enables temporal information about transcriptional or environmental histories potentially at single-cell resolution. By capturing temporal information about transcriptional histories, it could allow researchers to reconstruct dynamic microenvironments in bioreactors, track responses to different environmental conditions (e.g. nutrient limitations, pH-gradients) experienced by individual cells, the so-called microbial life-line (Blöbaum et al., 2023). The key advantage is that Record-seq preserves a molecular memory of cellular experiences, thereby providing insights into otherwise inaccessible fluctuations within large-scale systems.

Methodologically, Record-seq is a combined method of a CRISPR spacer acquisition with sequencing of the CRISPR array (Schmidt et al., 2018). It was demonstrated in *E. coli* that this recording allows to determine the characteristic gene expression histories that occur during the passing through a series of environmental events e.g. passing through the mammalian gut system (Schmidt et al., 2022). In contrast to RNA-seq that captures a transcriptional snapshot at one particular time point, a molecular recorder (based on a CRISPR system) allows to record the fast changing expression profiles and permanently store this as the transcriptional history as the cells autobiography (Schmidt, 2022). The transcriptional recording relies on a fusion protein comprising the reverse transcriptase domain (RT) and the Cas protein from *Fusicatenibacter saccharivorans* (Schmidt et al., 2018). New memories are stored by integrating new spacers ahead of old spacers within the CRISPR array and therefore building a memory of molecular events. Selective amplification of expanded CRISPR arrays' (SENECA) and deep sequencing allow identification of acquired spacers in the array.

Although Record-seq has not yet been applied to bioprocess-relevant questions, it offers a retrospective analysis that could in combination with scRNA-seq represent a behavioral fingerprinting in the context of the historical process conditions. This concept could be a powerful complement to spherical sensor-bead which are currently developed to report environmental profiles in bioreactors (Lauterbach et al., 2019). Thus, the information could guide process optimization or the genetic engineering of more robust cells. Lastly, one could imagine using Record-seq as complementary quality assessment attribute, when global bioprocess performance is decreasing to find potential biological bottlenecks.

However, Record-seq is still in its infancy for bioprocess applications and the potential bottlenecks have yet to be identified. Limitations may include recording capacity, potential biases in molecular editing mechanisms and the need to establish the technology in industrially relevant organisms prior to its application. Furthermore, interpreting spacer acquisition patterns into quantitative environmental histories demands a detailed characterization of e.g. the response time of the molecular recorder.

In conclusion, Record-seq holds promise as a high-risk, high-gain strategy that could bridge the gap between single-cell biology and bioprocess environments by uncovering hidden layers of cellular behavior. Nevertheless, Record-seq might be more relevant during the development of new processes and not suitable for routine application where bulk RNA-seq remains more feasible.

5. Outlook and further directions

Transcriptomics-driven progress was delayed for a long time in bioprocess engineering in comparison to molecular biology. Presumably due to the expensiveness of the transcriptomics data experimental workflows and missing analysis pipelines. Today, state-of-the-art

transcriptomics (RNA-seq and microarrays) are becoming increasingly attractive in bioprocess engineering for diverse research questions from the analysis of strain/cell line physiology to scale-up/down approaches as highlighted in this study.

The presented studies demonstrate the importance of transcriptomics-aided characterization of bioprocesses. The summarized works applied transcriptomics for a fundamental understanding of bioprocesses, implicating the potential to systematically use such methodologies for bioprocess optimization and/or control in the future. It can be recommended that the bioprocess engineering community should facilitate this technology with a systematic recording of transcriptome profiles from various bioreactor environments across different cellular production factories.

At the moment, transcriptomics-based bioprocess control is not possible as potential at-line application as such methods would simply be too slow and too expensive for industrial applications. First, direct RNA-sequencing would represent the fastest methods to get systemwide transcriptional information with estimated processing times of 3–4 h from sampling to quantitative transcriptome data. This is considered to be too long for efficient bioprocess control and does not allow real-time monitoring. For fast control loops, measurements signals should be measured continuously with sensor update rates in the range of seconds to minutes (Randek and Mandenius, 2018). RNA-seq, potentially reaching hour intervalls, could be used to monitor overall process performance, rather than used to trigger fast control loops. Second, current costs for direct RNA-sequencing via nanopore sequencing would most likely be too expensive for industrial application. Current estimates would be about 1,500 € per sample including library preparation and the PromethION flow cell. However, technological advancements with increased efficiencies, decreased processing time, and lowered prices might lead to concepts of transcriptome-based at-line monitoring and control in bioprocess engineering in the future. Once progress towards real-time monitoring RNA is achieved, the early stress gene expressions could trigger e.g. proactive adaptation of feed rates or oxygen supply before productivity drops.

Combining the above mentioned emerging transcriptional methods with conventional “omics” offers a huge change to use systematic biological data as an enabler to boost the development of innovative biotechnological processes. Guided by the concept of systems biology, the physiological behavior of cells/organisms in a bioprocess is determined by the regulation on each of the omics levels as well as their interlevel regulation that is shaped by the environment/bioreactor conditions. In theory, the combination of genomics, transcriptomics, proteomics, and metabolomics represents a powerful, however laborious and costly scenario, that holistically reveals an (industrial) bioprocess while identifying its bottlenecks (Becker and Wittmann, 2018). Mechanistic as well as data interference modelling approaches can aid in making those data actionable as limitations/bottlenecks can be identified giving new impetus for strain and bioprocess development (Masson et al., 2023). Genomics can be considered secondary as the number of generations remains low in typical (fed-)batch processes and under the assumption that the strain stability has been priviously validated. However, epigenetic signatures are becoming increasingly important as DNA modifications such as methylation are involved in gene expression without any changes in the genome sequence itself (Halabian et al., 2021). Transcriptomics alone will only be capable to identify bottlenecks that occur on the transcriptional level without any downstream limitations e.g. translational efficiency, protein stability, and feedback inhibition of enzymes. While the combination with any downstream omics method expands the repertoire of identifiable bottlenecks e.g. Ribo-seq for actively translated mRNA (Limbu et al., 2024) or quantitative proteomics for essential post-translational modifications (Yang and Qiao, 2023).

Emerging data analysis technologies are the key to extract meaningful data from the complexity of systemwide omics data (Tiwary, 2022). Exceeding the integration of machine learning approaches can support a better understanding of the complexity within the data. A blueprint could be the mining of genetic and transcriptional datasets as it is applied in personalized medicine that is developing rapidly (Su et al., 2020). Machine learning models have been developed to integrate e.g. transcriptome data in the context of biomedicine combined with further metadata to find new mechanisms and biomarkers. It can be envisioned by recording of both transcriptome profiles as well as metadata from the bioprocess (e.g. KPIs, process parameters) aided by automated workflows (Blums et al., 2025) that consequent processing of those data guided by machine learning and genome-scale metabolic models (Fang et al., 2020) has the power to unravel the complex trends across the transcriptional landscape of bioprocesses. While still at an early stage, these emerging tools provide a powerful foundation to bridge molecular and process-level insights, and it is expected that their integration will increasingly shape the future of data-driven bioprocess development and industrial application.

Funding sources

N.A.H. acknowledges funding by the Carl-Zeiss-Stiftung (Nexus Junior research group BIOSCALE).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

We acknowledge the use of the icons microtube-open-translucent icon by Servier https://smart.servier.com/ is licensed under CC-BY 3.0 Unported https://creativecommons.org/licenses/by/3.0/; rna icon by Servier https://smart.servier.com/ is licensed under CC-BY 3.0 Unported https://creativecommons.org/licenses/by/3.0/; genomesequencer9 icon by DBCLS https://togotv.dbcls.jp/en/pics.html is licensed under CC-BY 4.0 Unported https://creativecommons.org/licenses/by/4.0/; mortar-2 icon by DBCLS https://togotv.dbcls.jp/en/pics.html is licensed under CC-BY 4.0 Unportedhttps://creativecommons.org/licenses/by/4.0/. Readability and language style of the manuscript were supported using AI tools (ChatGTP and OpenAI), which were applied solely for linguistic refinement. All authors take full responsibility for the content.

Data availability

No data was used for the research described in the article.

References

Almogy, G., Pratt, M., Oberstrass, F., Lee, L., Mazur, D., Beckett, N., Barad, O., Soifer, I., Perelman, E., Etzioni, Y., Sosa, M., Jung, A., Clark, T., Trepagnier, E., Lithwick-Yanai, G., Pollock, S., Hornung, G., Levy, M., Coole, M., Howd, T., Shand, M., Farjoun, Y., Emery, J., Hall, G., Lee, S., Sato, T., Magner, R., Low, S., Bernier, A., Gandi, B., Stohlman, J., Nolet, C., Donovan, S., Blumenstiel, B., Cipicchio, M., Dodge, S., Banks, E., Lennon, N., Gabriel, S., Lipson, D., 2022. Cost-efficient whole genome-sequencing using novel mostly natural sequencing-by-synthesis chemistry and open fluidics platform. bioRxiv, 2022.2005.2029.493900.

Alsayyari, A.A., Pan, X., Dalm, C., van der Veen, J.W., Vriezen, N., Hageman, J.A., Wijffels, R.H., Martens, D.E., 2018. Transcriptome analysis for the scale-down of a CHO cell fed-batch process. J. Biotechnol. 279, 61–72.

Amer, B., Baidoo, E.E.K., 2021. Omics-driven biotechnology for industrial applications. Front. Bioeng. Biotechnol. 9, 613307.

Avello, V., Torres, M., Vergara, M., Berrios, J., Valdez-Cruz, N.A., Acevedo, C., Molina Sampayo, M., Dickson, A.J., Altamirano, C., 2022. Enhanced recombinant protein production in CHO cell continuous cultures under growth-inhibiting conditions is associated with an arrested cell cycle in G1/G0 phase. PLoS One 17 (11), e0277620.

Banerjee, D., Eng, T., Sasaki, Y., Srinivasan, A., Oka, A., Herbert, R.A., Trinh, J., Singan, V.R., Sun, N., Putnam, D., Scown, C.D., Simmons, B., Mukhopadhyay, A., 2021. Genomics characterization of an engineered *Corynebacterium glutamicum* in bioreactor cultivation under ionic liquid stress. Front. Bioeng. Biotechnol. 9, 766674.

Barzadd, M.M., Lundqvist, M., Harris, C., Malm, M., Volk, A.L., Thalén, N., Chotteau, V., Grassi, L., Smith, A., Abadi, M.L., Lambiase, G., Gibson, S., Hatton, D., Rockberg, J., 2022. Autophagy and intracellular product degradation genes identified by systems biology analysis reduce aggregation of bispecific antibody in CHO cells. New Biotechnol. 68, 68–76.

Baysoy, A., Bai, Z., Satija, R., Fan, R., 2023. The technological landscape and applications of single-cell multi-omics. Nat. Rev. Mol. Cell Biol. 24 (10), 695–713.

Becker, J., Wittmann, C., 2018. From systems biology to metabolically engineered cells-an omics perspective on the development of industrial microbes. Curr. Opin. Microbiol. 45, 180–188.

Blöbaum, L., Haringa, C., Grünberger, A., 2023. Microbial lifelines in bioprocesses: from concept to application. Biotechnol. Adv. 62, 108071.

Blums, K., Herzog, J., Costa, J., Quirico, L., Turber, J., Weuster-Botz, D., 2025. Automation of RNA-Seq sample preparation and miniaturized parallel bioreactors enable high-throughput differential gene expression studies. Microorganisms 13 (4), 849.

Boojari, M.A., Rajabi Ghaledari, F., Motamedian, E., Soleimani, M., Shojaosadati, S.A., 2023. Developing a metabolic model-based fed-batch feeding strategy for *Pichia pastoris* fermentation through fine-tuning of the methanol utilization pathway. Microb. Biotechnol. 16 (6), 1344–1359.

Buccitelli, C., Selbach, M., 2020. mRNAs, proteins and the emerging principles of gene expression control. Nat. Rev. Genet. 21 (10), 630–644.

Buchholz, J., Graf, M., Freund, A., Busche, T., Kalinowski, J., Blombach, B., Takors, R., 2014. CO_2 /HCO_3^- perturbations of simulated large scale gradients in a scale-down device cause fast transcriptional responses in *Corynebacterium glutamicum*. Appl. Microbiol. Biotechnol. 98 (20), 8563–8572.

Bui, T.T., Selvarajoo, K., 2020. Attractor concepts to evaluate the transcriptome-wide dynamics guiding anaerobic to aerobic state transition in *Escherichia coli*. Sci. Rep. 10 (1), 5878.

Bumgarner, R., 2013. Overview of DNA microarrays: types, applications, and their future. Curr. Protoc. Mol. Biol. 101 (1), 22.21.21-22.21.11.

Cankar, K., Henke, N.A., Wendisch, V.F., 2023. Functional food additives/ingredients production by engineered *Corynebacterium glutamicum*. Syst. Microbiol. Biomanuf. 3 (1), 110–121.

Chen, W., Guillaume-Gentil, O., Rainer, P.Y., Gäbelein, C.G., Saelens, W., Gardeux, V., Klaeger, A., Dainese, R., Zachara, M., Zambelli, T., Vorholt, J.A., Deplancke, B., 2022. Live-seq enables temporal transcriptomic recording of single cells. Nature 608 (7924), 733–740.

Cortada-Garcia, J., Haggarty, J., Moses, T., Daly, R., Arnold, S.A., Burgess, K., 2022. On-line untargeted metabolomics monitoring of an *Escherichia coli* succinate fermentation process. Biotechnol. Bioeng. 119 (10), 2757–2769.

Cortada-Garcia, J., Haggarty, J., Weidt, S., Daly, R., Arnold, S.A., Burgess, K., 2024. On-line targeted metabolomics for real-time monitoring of relevant compounds in fermentation processes. Biotechnol. Bioeng. 121 (2), 683–695.

De Jonghe, J., Opzoomer, J.W., Vilas-Zornoza, A., Nilges, B.S., Crane, P., Vicari, M., Lee, H., Lara-Astiaso, D., Gross, T., Morf, J., Schneider, K., Cudini, J., Ramos-Mucci, L., Mooijman, D., Tiklová, K., Salas, S.M., Langseth, C.M., Kashikar, N.D., Carrami, E.M., McIntyre, R., Swerner, C.B., Hessel, E.M., Kapourani, C.-A., Regep, C., Roberts, C.E.S., Schapiro, D., Lundeberg, J., Nilsson, M., Shalek, A.K., Cribbs, A.P., Taylor-King, J.P., 2024. scTrends: a living review of commercial single-cell and spatial 'omic technologies. Cell Genomics 4 (12).

Delvigne, F., Goffin, P., 2014. Microbial heterogeneity affects bioprocess robustness: dynamic single-cell analysis contributes to understanding of microbial populations. Biotechnol. J. 9 (1), 61–72.

DeRisi, J.L., Iyer, V.R., Brown, P.O., 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. Science 278 (5338), 680–686.

Doran, P.M., 1995. In: Doran, Pauline M. (Ed.), Bioprocess engineering principles. Academic Press, London; San Diego [1995] ©1995.

Dostálová, H., Busche, T., Holátko, J., Rucká, L., Štěpánek, V., Barvík, I., Nešvera, J., Kalinowski, J., Pátek, M., 2018. Overlap of promoter recognition specificity of stress response sigma factors SigD and SigH in *Corynebacterium glutamicum* ATCC 13032. Front. Microbiol. 9, 3287.

Fang, X., Lloyd, C.J., Palsson, B.O., 2020. Reconstructing organisms in silico: genome-scale models and their emerging applications. Nat. Rev. Microbiol. 18 (12), 731–743.

Feng, X., Page, L., Rubens, J., Chircus, L., Colletti, P., Pakrasi, H.B., Tang, Y.J., 2010. Bridging the gap between fluxomics and industrial biotechnology. J. Biomed. Biotechnol. 2010, 460717.

Fenouil, R., Pradel, N., Belahbib, H., Roumagnac, M., Bartoli, M., Ben Hania, W., Denis, Y., Garel, M., Tamburini, C., Ollivier, B., Summers, Z., Armougom, F., Dolla, A., 2023. Adaptation strategies to high hydrostatic pressures in *Pseudothermotoga* species revealed by transcriptional analyses. Microorganisms 11 (3).

Fletcher, E., Krivoruchko, A., Nielsen, J., 2016. Industrial systems biology and its impact on synthetic biology of yeast cell factories. Biotechnol. Bioeng. 113 (6), 1164–1170.

Fulcher, J.M., Markillie, L.M., Mitchell, H.D., Williams, S.M., Engbrecht, K.M., Degnan, D.J., Bramer, L.M., Moore, R.J., Chrisler, W.B., Cantlon-Bruce, J., Bagnoli, J.W., Qian, W.-J., Seth, A., Paša-Tolić, L., Zhu, Y., 2024. Parallel measurement of transcriptomes and proteomes from same single cells using nanodroplet splitting. Nat. Commun. 15 (1), 10614.

Garalde, D.R., Snell, E.A., Jachimowicz, D., Sipos, B., Lloyd, J.H., Bruce, M., Pantic, N., Admassu, T., James, P., Warland, A., Jordan, M., Ciccone, J., Serra, S., Keenan, J., Martin, S., McNeill, L., Wallace, E.J., Jayasinghe, L., Wright, C., Blasco, J., Young, S., Brocklebank, D., Juul, S., Clarke, J., Heron, A.J., Turner, D.J., 2018. Highly parallel direct RNA sequencing on an array of nanopores. Nat. Methods 15 (3), 201–206.

Gareau, T., Lara, G.G., Shepherd, R.D., Krawetz, R., Rancourt, D.E., Rinker, K.D., Kallos, M.S., 2014. Shear stress influences the pluripotency of murine embryonic stem cells in stirred suspension bioreactors. J. Tissue Eng. Regen. Med. 8 (4), 268–278.

Graf, M., Haas, T., Teleki, A., Feith, A., Cerff, M., Wiechert, W., Nöh, K., Busche, T., Kalinowski, J., Takors, R., 2020. Revisiting the growth modulon of *Corynebacterium glutamicum* under glucose limited chemostat conditions. Front. Bioeng. Biotechnol. 8, 584614.

Grassi, L., Harris, C., Zhu, J., Hatton, D., Dunn, S., 2025. Next-generation sequencing: a powerful multi-purpose tool in cell line development for biologics production. Comput. Struct. Biotechnol. J. 27, 1511–1517.

Greenbaum, D., Colangelo, C., Williams, K., Gerstein, M., 2003. Comparing protein abundance and mRNA expression levels on a genomic scale. Genome Biol. 4 (9), 117.

Grünberger, A., Wiechert, W., Kohlheyer, D., 2014. Single-cell microfluidics: opportunity for bioprocess development. Curr. Opin. Biotechnol. 29, 15–23.

Gupta, P., Lee, K.H., 2007. Genomics and proteomics in process development: opportunities and challenges. Trends Biotechnol. 25 (7), 324–330.

Haas, T., Graf, M., Nieß, A., Busche, T., Kalinowski, J., Blombach, B., Takors, R., 2019. Identifying the growth modulon of *Corynebacterium glutamicum*. Front. Microbiol. 10, 974.

Halabian, R., Valizadeh, A., Ahmadi, A., Saeedi, P., Azimzadeh Jamalkandi, S., Alivand, M.R., 2021. Laboratory methods to decipher epigenetic signatures: a comparative review. Cell. Mol. Biol. Lett. 26 (1), 46.

Hasni, I., Decloquement, P., Demanèche, S., Mameri, R.M., Abbe, O., Colson, P., La Scola, B., 2020. Insight into the lifestyle of amoeba *Willaertia magna* during bioreactor growth using transcriptomics and proteomics. Microorganisms 8 (5).

Heins, A.L., Hoang, M.D., Weuster-Botz, D., 2022. Advances in automated real-time flow cytometry for monitoring of bioreactor processes. Eng. Life Sci. 22 (3–4), 260–278.

Heumos, L., Schaar, A.C., Lance, C., Litinetskaya, A., Drost, F., Zappia, L., Lücken, M.D., Strobl, D.C., Henao, J., Curion, F., Aliee, H., Ansari, M., Badia-i-Mompel, P., Büttner, M., Dann, E., Dimitrov, D., Dony, L., Frishberg, A., He, D., Hediyeh-zadeh, S., Hetzel, L., Ibarra, I.L., Jones, M.G., Lotfollahi, M., Martens, L.D., Müller, C. L., Nitzan, M., Ostner, J., Palla, G., Patro, R., Piran, Z., Ramírez-Suástegui, C., Saez-Rodriguez, J., Sarkar, H., Schubert, B., Sikkema, L., Srivastava, A., Tanevski, J., Virshup, I., Weiler, P., Schiller, H.B., Theis, F.J., Single-cell Best Practices, C, 2023. Best practices for single-cell analysis across modalities. Nat. Rev. Genet. 24 (8), 550–572.

Hoheisel, J.D., 1997. Oligomer-chip technology. Trends Biotechnol. 15 (11), 465–469.

Homberger, C., Barquist, L., Vogel, J., 2022. Ushering in a new era of single-cell transcriptomics in bacteria. microLife 3.

Houser, J.R., Barnhart, C., Boutz, D.R., Carroll, S.M., Dasgupta, A., Michener, J.K., Needham, B.D., Papoulas, O., Sridhara, V., Sydykova, D.K., Marx, C.J., Trent, M.S., Barrick, J.E., Marcotte, E.M., Wilke, C.O., 2015. Controlled measurement and comparative analysis of cellular components in *E. coli* reveals broad regulatory changes in response to glucose starvation. PLoS Comput. Biol. 11 (8), e1004400.

Inayatullah, M., Dwivedi, A.K., Tiwari, V.K., 2025. Advances in single-cell omics: transformative applications in basic and clinical research. Curr. Opin. Cell Biol. 95, 102548.

Jayapal, K.P., Goudar, C.T., 2014. Transcriptomics as a tool for assessing the scalability of mammalian cell perfusion systems. Adv. Biochem. Eng. Biotechnol. 139, 227–243.

Käß, F., Hariskos, I., Michel, A., Brandt, H.J., Spann, R., Junne, S., Wiechert, W., Neubauer, P., Oldiges, M., 2014. Assessment of robustness against dissolved oxygen/substrate oscillations for *C. glutamicum* DM1933 in two-compartment bioreactor. Bioprocess Biosyst. Eng. 37 (6), 1151–1162.

Kerssemakers, A.A.J., Øzmerih, S., Sin, G., Sudarsan, S., 2023. Dynamic interplay between O2 availability, growth rates, and the transcriptome of *Yarrowia lipolytica*. Fermentation 9 (1), 74.

Kim, M., Park, B.G., Kim, J., Kim, J.Y., Kim, B.-G., 2018. Exploiting transcriptomic data for metabolic engineering: toward a systematic strain design. Curr. Opin. Biotechnol. 54, 26–32.

Klausing, S., Krämer, O., Noll, T., 2011. Bioreactor cultivation of CHO DP-12 cells under sodium butyrate treatment - comparative transcriptome analysis with CHO cDNA microarrays. BMC Proc. 5 Suppl 8(Suppl 8), P98.

Kokoris, M., McRuer, R., Nabavi, M., Jacobs, A., Prindle, M., Cech, C., Berg, K., Lehmann, T., Machacek, C., Tabone, J., Chandrasekar, J., McGee, L., Lopez, M., Reid, T., Williams, C., Barrett, S., Lehmann, A., Kovarik, M., Busam, R., Miller, S., Banasik, B., Kesic, B., Arryman, A., Rogers-Peckham, M., Kimura, A., LeProwse, M., Wolfin, M., Kritzer, S., Leadbetter, J., Babazadeh, M., Chase, J., Thiessen, G., Lint, W., Goodman, D., O'Connell, D., Lumanpauw, N., Hoffman, J., Vellucci, S., Collins, K., Vellucci, J., Taylor, A., Murphy, M., Lee, M., Corning, M., 2025. Sequencing by Expansion (SBX) – a novel, high-throughput single-molecule sequencing technology bioRxiv, 2025.2002.2019.639056.

Kondragunta, B., Joshi, B.H., Han, J., Brorson, K.A., Puri, R.K., Moreira, A.R., Rao, G., 2012. Bioreactor environment-sensitive sentinel genes as novel metrics for cell culture scale-down comparability. Biotechnol. Prog. 28 (5), 1138–1151.

Küberl, A., Mengus-Kaya, A., Polen, T., Bott, M., 2020. The iron deficiency response of *Corynebacterium glutamicum* and a link to thiamine biosynthesis. Appl. Environ. Microbiol. 86 (10).

Kulkarni, A., Anderson, A.G., Merullo, D.P., Konopka, G., 2019. Beyond bulk: a review of single cell transcriptomics methodologies and applications. Curr. Opin. Biotechnol. 58, 129–136.

Lara, A.R., Galindo, E., Ramírez, O.T., Palomares, L.A., 2006a. Living with heterogeneities in bioreactors: understanding the effects of environmental gradients on cells. Mol. Biotechnol. 34 (3), 355–381.

Lara, A.R., Leal, L., Flores, N., Gosset, G., Bolívar, F., Ramírez, O.T., 2006b. Transcriptional and metabolic response of recombinant *Escherichia coli* to spatial dissolved oxygen tension gradients simulated in a scale-down system. Biotechnol. Bioeng. 93 (2), 372–385.

Lauterbach, T., Lüke, T., Büker, M.-J., Hedayat, C., Gernandt, T., Moll, R., Grösel, M., Lenk, S., Seidel, F., Brunner, D., Bley, T., Walther, T., Lenk, F., 2019. Measurements on the fly– introducing mobile micro-sensors for biotechnological applications. Sensors Actuators A Phys. 287, 29–38.
Limbu, M.S., Xiong, T., Wang, S., 2024. A review of ribosome profiling and tools used in Ribo-seq data analysis. Comput. Struct. Biotechnol. J. 23, 1912–1918.
Liu-Wei, W., van der Toorn, W., Bohn, P., Hölzer, M., Smyth, R.P., von Kleist, M., 2024. Sequencing accuracy and systematic errors of nanopore direct RNA sequencing. BMC Genomics 25 (1), 528.
Lockhart, D.J., Dong, H., Byrne, M.C., Follettie, M.T., Gallo, M.V., Chee, M.S., Mittmann, M., Wang, C., Kobayashi, M., Norton, H., 1996. Expression monitoring by hybridization to high-density oligonucleotide arrays. Nat. Biotechnol. 14 (13), 1675–1680.
Löffler, M., Simen, J.D., Jäger, G., Schäferhoff, K., Freund, A., Takors, R., 2016. Engineering *E. coli* for large-scale production – strategies considering ATP expenses and transcriptional responses. Metab. Eng. 38, 73–85.
Ma, P., Amemiya, H.M., He, L.L., Gandhi, S.J., Nicol, R., Bhattacharyya, R.P., Smillie, C. S., Hung, D.T., 2023. Bacterial droplet-based single-cell RNA-seq reveals antibiotic-associated heterogeneous cellular states. Cell 186 (4), 877–891.e814.
Maccani, A., Hackl, M., Leitner, C., Steinfellner, W., Graf, A.B., Tatto, N.E., Karbiener, M., Scheideler, M., Grillari, J., Mattanovich, D., Kunert, R., Borth, N., Grabherr, R., Ernst, W., 2014. Identification of microRNAs specific for high producer CHO cell lines using steady-state cultivation. Appl. Microbiol. Biotechnol. 98 (17), 7535–7548.
Maier, T., Güell, M., Serrano, L., 2009. Correlation of mRNA and protein in complex biological samples. FEBS Lett. 583 (24), 3966–3973.
Marguerat, S., Bähler, J., 2010. RNA-seq: from technology to biology. Cell. Mol. Life Sci. 67 (4), 569–579.
Marison, I., Hennessy, S., Foley, R., Schuler, M., Sivaprakasam, S., Freeland, B., 2013. The choice of suitable online analytical techniques and data processing for monitoring of bioprocesses. In: Mandenius, C.-F., Titchener-Hooker, N.J. (Eds.), Measurement, Monitoring, Modelling and Control of Bioprocesses. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp. 249–280.
Masson, H.O., Karottki, K.J.C., Tat, J., Hefzi, H., Lewis, N.E., 2023. From observational to actionable: rethinking omics in biologics production. Trends Biotechnol. 41 (9), 1127–1138.
Menke, S., Gillingham, M.A., Wilhelm, K., Sommer, S., 2017. Home-made cost effective preservation buffer is a better alternative to commercial preservation methods for microbiome research. Front. Microbiol. 8, 102.
Messner, C.B., Demichev, V., Wang, Z., Hartl, J., Kustatscher, G., Mülleder, M., Ralser, M., 2023. Mass spectrometry-based high-throughput proteomics and its role in biomedical studies and systems biology. PROTEOMICS 23 (7–8), 2200013.
Metzker, M.L., 2010. Sequencing technologies — the next generation. Nat. Rev. Genet. 11 (1), 31–46.
Miller, D.C., Athavale, S.V., Arnold, F.H., 2022. Combining chemistry and protein engineering for new-to-nature biocatalysis. Nat Synth 1 (1), 18–23.
Minden, S., Aniolek, M., Noorman, H., Takors, R., 2023. Mimicked mixing-induced heterogeneities of industrial bioreactors stimulate long-lasting adaption programs in ethanol-producing yeasts. Genes (Basel) 14 (5).
Moon, J.H., Roh, D.H., Kwack, K.H., Lee, J.H., 2023. Bacterial single-cell transcriptomics: recent technical advances and future applications in dentistry. Jpn. Dent. Sci. Rev. 59, 253–262.
Neubauer, P., Cruz, N., Glauche, F., Junne, S., Knepper, A., Raven, M., 2013. Consistent development of bioprocesses from microliter cultures to the industrial scale. Eng. Life Sci. 13 (3), 224–238.
Nitsche, B.M., Jørgensen, T.R., Akeroyd, M., Meyer, V., Ram, A.F., 2012. The carbon starvation response of *Aspergillus niger* during submerged cultivation: insights from the transcriptome and secretome. BMC Genomics 13, 380.
Oldiges, M., Lütz, S., Pflug, S., Schroer, K., Stein, N., Wiendahl, C., 2007. Metabolomics: current state and evolving methodologies and tools. Appl. Microbiol. Biotechnol. 76 (3), 495–511.
Otero, J.M., Nielsen, J., 2010. Industrial systems biology. Biotechnol. Bioeng. 105 (3), 439–460.
Prielhofer, R., Cartwright, S.P., Graf, A.B., Valli, M., Bill, R.M., Mattanovich, D., Gasser, B., 2015. *Pichia pastoris* regulates its gene-specific response to different carbon sources at the transcriptional, rather than the translational, level. BMC Genomics 16 (1), 167.
Prondzynski, M., Berkson, P., Trembley, M.A., Tharani, Y., Shani, K., Bortolin, R.H., Sweat, M.E., Mayourian, J., Yucel, D., Cordoves, A.M., Gabbin, B., Hou, C., Anyanwu, N.J., Nawar, F., Cotton, J., Milosh, J., Walker, D., Zhang, Y., Lu, F., Liu, X., Parker, K.K., Bezzerides, V.J., Pu, W.T., 2024. Efficient and reproducible generation of human iPSC-derived cardiomyocytes and cardiac organoids in stirred suspension systems. Nat. Commun. 15 (1), 5929.
Pucker, B., Schilbert, H.M., 2019. Genomics and transcriptomics advance in plant sciences. In: Singh, S.P., Upadhyay, S.K., Pandey, A., Kumar, S. (Eds.), Molecular Approaches in Plant Biology and Environmental Challenges. Springer Singapore, Singapore, pp. 419–448.
Pucker, B., Irisarri, I., de Vries, J., Xu, B., 2022. Plant genome sequence assembly in the era of long reads: Progress, challenges and future directions. Quant. Plant Biol. 3, e5.
Randek, J., Mandenius, C.F., 2018. On-line soft sensing in upstream bioprocessing. Crit. Rev. Biotechnol. 38 (1), 106–121.
Reiter, A., Herbst, L., Wiechert, W., Oldiges, M., 2021. Need for speed: evaluation of dilute and shoot-mass spectrometry for accelerated metabolic phenotyping in bioprocess development. Anal. Bioanal. Chem. 413 (12), 3253–3268.
Rodrigues, K.C.S., Sonego, J.L.S., Bernardo, A., Ribeiro, M.P.A., Cruz, A.J.G., Badino, A. C., 2018. Real-time monitoring of bioethanol fermentation with industrial musts using mid-infrared spectroscopy. Ind. Eng. Chem. Res. 57 (32), 10823–10831.
Rohani, L., Borys, B.S., Razian, G., Naghsh, P., Liu, S., Johnson, A.A., Machiraju, P., Holland, H., Lewis, I.A., Groves, R.A., Toms, D., Gordon, P.M.K., Li, J.W., So, T., Dang, T., Kallos, M.S., Rancourt, D.E., 2020. Stirred suspension bioreactors maintain naïve pluripotency of human pluripotent stem cells. Commun. Biol. 3 (1), 492.
Sauer, M., Branduardi, P., Gasser, B., Valli, M., Maurer, M., Porro, D., Mattanovich, D., 2004. Differential gene expression in recombinant *Pichia pastoris* analysed by heterologous DNA microarray hybridisation. Microb. Cell Factories 3 (1), 17.
Schena, M., Shalon, D., Davis, R.W., Brown, P.O., 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270 (5235), 467–470.
Schmidt, F., 2022. Autobiography of a gut bacterium. Science 378 (6622), 844–845.
Schmidt, F., Cherepkova, M.Y., Platt, R.J., 2018. Transcriptional recording by CRISPR spacer acquisition from RNA. Nature 562 (7727), 380–385.
Schmidt, F., Zimmermann, J., Tanna, T., Farouni, R., Conway, T., Macpherson, A.J., Platt, R.J., 2022. Noninvasive assessment of gut function using transcriptional recording sentinel cells. Science 376 (6594) eabm6038.
Schulze, M., Kumar, Y., Rattay, M., Niemann, J., Wijffels, R.H., Martens, D.E., 2022. Transcriptomic analysis reveals mode of action of butyric acid supplementation in an intensified CHO cell fed-batch process. Biotechnol. Bioeng. 119 (9), 2359–2373.
Schweder, T., Krüger, E., Xu, B., Jürgen, B., Blomsten, G., Enfors, S.O., Hecker, M., 1999. Monitoring of genes that respond to process-related stress in large- scale bioprocesses. Biotechnol. Bioeng. 65 (2), 151–159.
Selinger, D.W., Cheung, K.J., Mei, R., Johansson, E.M., Richmond, C.S., Blattner, F.R., Lockhart, D.J., Church, G.M., 2000. RNA expression analysis using a 30 base pair resolution *Escherichia coli* genome array. Nat. Biotechnol. 18 (12), 1262–1268.
Sharon, D., Tilgner, H., Grubert, F., Snyder, M., 2013. A single-molecule long-read survey of the human transcriptome. Nat. Biotechnol. 31 (11), 1009–1014.
Shekhawat, K., Patterton, H., Bauer, F.F., Setati, M.E., 2019. RNA-seq based transcriptional analysis of *Saccharomyces cerevisiae* and Lachancea thermotolerans in mixed-culture fermentations under anaerobic conditions. BMC Genomics 20 (1), 145.
Shilpha, J., Lee, J., Kwon, J.-S., Lee, H.-A., Nam, J.-Y., Jang, H., Kang, W.-H., 2024. An improved bacterial mRNA enrichment strategy in dual RNA sequencing to unveil the dynamics of plant-bacterial interactions. Plant Methods 20 (1), 99.
Sieck, J.B., Cordes, T., Budach, W.E., Rhiel, M.H., Suemeghy, Z., Leist, C., Villiger, T.K., Morbidelli, M., Soos, M., 2013. Development of a scale-down model of hydrodynamic stress to study the performance of an industrial CHO cell line under simulated production scale bioreactor conditions. J. Biotechnol. 164 (1), 41–49.
Simen, J.D., Löffler, M., Jäger, G., Schäferhoff, K., Freund, A., Matthes, J., Müller, J., Takors, R., 2017. Transcriptional response of *Escherichia coli* to ammonia and glucose fluctuations. Microb. Biotechnol. 10 (4), 858–872.
Singh, R., Fatima, E., Thakur, L., Singh, S., Ratan, C., Kumar, N., 2024. Advancements in CHO metabolomics: techniques, current state and evolving methodologies. Front. Bioeng. Biotechnol. 12, 1347138.
Su, C., Tong, J., Wang, F., 2020. Mining genetic and transcriptomic data using machine learning approaches in Parkinson's disease. npj Parkinson's Dis. 6 (1), 24.
Tanaka, K., Bamba, T., Kondo, A., Hasunuma, T., 2024. Metabolomics-based development of bioproduction processes toward industrial-scale production. Curr. Opin. Biotechnol. 85, 103057.
Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B.B., Siddiqui, A., Lao, K., Surani, M.A., 2009. mRNA-Seq whole-transcriptome analysis of a single cell. Nat. Methods 6 (5), 377–382.
Tang, F., Barbacioru, C., Nordman, E., Li, B., Xu, N., Bashkirov, V.I., Lao, K., Surani, M. A., 2010. RNA-Seq analysis to capture the transcriptome landscape of a single cell. Nat. Protoc. 5 (3), 516–535.
Tang, F., Lao, K., Surani, M.A., 2011. Development and applications of single-cell transcriptome analysis. Nat. Methods 8 (4), S6–S11.
Tanna, T., Schmidt, F., Cherepkova, M.Y., Okoniewski, M., Platt, R.J., 2020. Recording transcriptional histories using Record-seq. Nat. Protoc. 15 (2), 513–539.
ter Linde, J.J., Liang, H., Davis, R.W., Steensma, H.Y., van Dijken, J.P., Pronk, J.T., 1999. Genome-wide transcriptional analysis of aerobic and anaerobic chemostat cultures of *Saccharomyces cerevisiae*. J. Bacteriol. 181 (24), 7409–7413.
Tiwary, B.K., 2022. Systems biology. In: Tiwary, B.K. (Ed.), Bioinformatics and Computational Biology: A Primer for Biologists. Springer Singapore, Singapore, pp. 137–162.
UN General, A., 2015. In: Assembly, U.G. (Ed.), Transforming Our World : the 2030 Agenda for Sustainable Development.
Unthan, S., Grunberger, A., van Ooyen, J., Gatgens, J., Heinrich, J., Paczia, N., Wiechert, W., Kohlheyer, D., Noack, S., 2014. Beyond growth rate 0.6: what drives *Corynebacterium glutamicum* to higher growth rates in defined medium. Biotechnol. Bioeng. 111 (2), 359–371.
van Duuren, J.B., Puchałka, J., Mars, A.E., Bücker, R., Eggink, G., Wittmann, C., Dos Santos, V.A., 2013. Reconciling in vivo and in silico key biological parameters of *Pseudomonas putida* KT2440 during growth on glucose under carbon-limited condition. BMC Biotechnol. 13, 93.
van Vliet, A.H., 2010. Next generation sequencing of microbial transcriptomes: challenges and opportunities. FEMS Microbiol. Lett. 302 (1), 1–7.
Vodopivec, M., Lah, L., Narat, M., Curk, T., 2019. Metabolomic profiling of CHO fed-batch growth phases at 10, 100, and 1,000 L. Biotechnol. Bioeng. 116 (10), 2720–2729.
von Stockar, U., Valentinotti, S., Marison, I., Cannizzaro, C., Herwig, C., 2003. Know-how and know-why in biochemical engineering. Biotechnol. Adv. 21 (5), 417–430.

Vries, A.R.G.D., Pronk, J.T., Daran, J.-M.G., 2017. Industrial relevance of chromosomal copy number variation in *Saccharomyces* yeasts. Appl. Environ. Microbiol. 83 (11), e03206–e03216.
Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet. 10 (1), 57–63.
Wang, Y., Liu, J., Ni, X., Lei, Y., Zheng, P., Diao, A., 2018. Screening efficient constitutive promoters in *Corynebacterium glutamicum* based on time-series transcriptome analysis. Sheng Wu Gong Cheng Xue Bao 34 (11), 1760–1771.
Wang, H., Chen, Y., Yang, Z., Deng, H., Liu, Y., Wei, P., Zhu, Z., Jiang, L., 2024. Metabolic and bioprocess engineering of *Clostridium tyrobutyricum* for butyl butyrate production on xylose and shrimp shell waste. Foods (Basel, Switzerland) 13 (7).
Wendisch, V.F., 2003. Genome-wide expression analysis in *Corynebacterium glutamicum* using DNA microarrays. J. Biotechnol. 104 (1–3), 273–285.
Wewetzer, S.J., Kunze, M., Ladner, T., Luchterhand, B., Roth, S., Rahmen, N., Kloß, R., Costa e Silva, A., Regestein, L., Büchs, J., 2015. Parallel use of shake flask and microtiter plate online measuring devices (RAMOS and BioLector) reduces the number of experiments in laboratory-scale stirred tank bioreactors. J. Biol. Eng. 9 (1), 9.
Wiechert, W., Schweissgut, O., Takanaga, H., Frommer, W.B., 2007. Fluxomics: mass spectrometry versus quantitative imaging. Curr. Opin. Plant Biol. 10 (3), 323–330.
Wiechert, W., Scheper, T., Weuster-Botz, D., 2022. Taking Biotech Research to the Next Level.
Wright, N.R., Rønnest, N.P., Sonnenschein, N., 2020. Single-cell technologies to understand the mechanisms of cellular adaptation in chemostats. Front. Bioeng. Biotechnol. 8.
Wu, Y., Lei, S., Lu, C., Li, J., Du, G., Liu, Y., 2023. Enhanced ribonucleic acid production by high-throughput screening based on fluorescence activation and transcriptomic-guided fermentation optimization in *Saccharomyces cerevisiae*. J. Agric. Food Chem. 71 (17), 6673–6680.
Wurm, M.J., Wurm, F.M., 2021. Naming CHO cells for bio-manufacturing: genome plasticity and variant phenotypes of cell populations in bioreactors question the relevance of old names. Biotechnol. J. 16 (7), 2100165.
Xu, M., Vallières, C., Finnis, C., Winzer, K., Avery, S.V., 2024. Exploiting phenotypic heterogeneity to improve production of glutathione by yeast. Microb. Cell Factories 23 (1), 267.
Yang, Y., Qiao, L., 2023. Data-independent acquisition proteomics methods for analyzing post-translational modifications. PROTEOMICS 23 (7–8), 2200046.
Yu, S., Guo, M., Zhang, Y., Bo, C., Liang, H., Wang, H., Yang, X., 2022. Comparative multiomics analysis of cell physiological state after culture in a basket bioreactor. Sci. Rep. 12 (1), 20161.
Yunus, I.S., Lee, T.S., 2022. Applications of targeted proteomics in metabolic engineering: advances and opportunities. Curr. Opin. Biotechnol. 75, 102709.
Zhang, C., Ma, Y., Miao, H., Tang, X., Xu, B., Wu, Q., Mu, Y., Huang, Z., 2020. Transcriptomic analysis of *Pichia pastoris (Komagataella phaffii)* GS115 during heterologous protein production using a high-cell-density fed-batch cultivation strategy. Front. Microbiol. 11, 463.
Zhang, M., Gao, Y., Yu, C., Wang, J., Weng, K., Li, Q., He, Y., Guo, Z., Zhang, H., Huang, J., Li, L., 2022. Transcriptome analysis of malate-induced *Schizochytrium* sp. FJU-512 reveals a novel pathway for biosynthesis of docosahexaenoic acid with enhanced expression of genes responsible for acetyl-CoA and NADPH accumulation. Front. Microbiol. 13, 1006138.
Zhao, Z., Cai, M., Liu, Y., Hu, M., Yang, F., Zhu, R., Xu, M., Rao, Z., 2022. Genomics and transcriptomics-guided metabolic engineering *Corynebacterium glutamicum* for l-arginine production. Bioresour. Technol. 364, 128054.
Zhou, S., Ding, N., Han, R., Deng, Y., 2023. Metabolic engineering and fermentation optimization strategies for producing organic acids of the tricarboxylic acid cycle by microbial cell factories. Bioresour. Technol. 379, 128986.
Ziegler, M., Zieringer, J., Döring, C.L., Paul, L., Schaal, C., Takors, R., 2021a. Engineering of a robust *Escherichia coli* chassis and exploitation for large-scale production processes. Metab. Eng. 67, 75–87.
Ziegler, M., Zieringer, J., Takors, R., 2021b. Transcriptional profiling of the stringent response mutant strain *E. coli* SR reveals enhanced robustness to large-scale conditions. Microb. Biotechnol. 14 (3), 993–1010.
Zu, T.N.K., Liu, S., Gerlach, E.S., Germane, K.L., Servinsky, M.D., Mackie, D.M., Sund, C. J., 2017. Real-time metabolite monitoring of glucose-fed *Clostridium acetobutylicum* fermentations using Raman assisted metabolomics. J. Raman Spectrosc. 48 (12), 1852–1862.