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Research review paper

Microbial lifelines in bioprocesses: From concept to application

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ABSTRACT

Bioprocesses are scaled up for the production of large product quantities. With larger fermenter volumes, mixing becomes increasingly inefficient and environmental gradients get more prominent than in smaller scales. Environmental gradients have an impact on the microorganism's metabolism, which makes the prediction of large-scale performance difficult and can lead to scale-up failure. A promising approach for improved understanding and estimation of dynamics of microbial populations in large-scale bioprocesses is the analysis of microbial lifelines. The lifeline of a microbe in a bioprocess is the experience of environmental gradients from a cell's perspective, which can be described as a time series of position, environment and intracellular condition. Currently, lifelines are predominantly determined using models with computational fluid dynamics, but new technical developments in flow-following sensor particles and microfluidic single-cell cultivation open the door to a more interdisciplinary concept. We critically review the current concepts and challenges in lifeline determination and application of lifeline analysis, as well as strategies for the integration of these techniques into bioprocess development. Lifelines can contribute to a successful scale-up by guiding scale-down experiments and identifying strain engineering targets or bioreactor optimisations.

1. Introduction

Microbes in their natural habitat experience continuously changing environments. The amplitude and frequency of the environmental changes depend on the habitat, for example soil, water or the body (Nguyen et al., 2021b). Microorganisms are adapted to cope with the dynamics in their natural environments. In biotechnology, they are introduced to an artificial environment: the bioreactor.

In the bioreactor, the microbial population is kept in well controlled conditions for the production of biotechnological products like high values molecules. Scale-up of bioprocesses to large volumes enables the production of large product quantities. Typical industrial-scale stirred tank reactors (STR) and bubble columns have volumes in the range of 50–500 m^3 (Fackler et al., 2021) though larger volumes may be encountered.

The scale-up of a bioprocess from laboratory scale to production scale (Fig. 1) is a challenge with the risk of affecting the efficiency and profitability of the process (Crater and Lievense, 2018; Lara et al., 2006). One phenomenon observed during scale-up is the formation of environmental gradients in large-scale bioreactors, which are less profound in lab-scale bioreactors. Nutrients, oxygen, control agents and temperature are distributed in the fermentation broth by mixing. When moving from small to large volumes (Fig. 1), mixing to reach the same degree of homogeneity in the same time becomes increasingly impractical, as the needed power input scales faster than the circulation time (Lara et al., 2006). This discrepancy results in longer mixing times in large-scale bioreactors, which can easily increase to more than 2 min for 95% homogeneity (Vrábel et al., 2000). The microorganisms experience the

Abbreviations: ATP, adenosine triphosphate; CFD, computational fluid dynamics; C_s, local nutrient concentration; dMSCC, dynamic microfluidic single-cell cultivation; DO, dissolved oxygen; K_S, substrate specific uptake constant; LB-LES, Lattice-Boltzmann Large Eddy Simulation; LL, Lifelines; μ, specific growth rate; (m)RNA, (messenger) ribonucleic acid; NAD(P)H, nicotinamide adenine dinucleotide (phosphate); PDMS, polydimethylsiloxane; PEP, phosphoenolpyruvate; PYR, pyruvate; q_s, specific uptake rate; r, reaction rate; RPT, radioactive particle tracking; STR, stirred tank reactor; $Y_{P/X}$, biomass specific product yield; $Y_{X/S}$, substrate specific biomass yield.

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resulting process parameter gradients in the bioreactor and need to adapt accordingly, which leads to increased metabolic costs (Minden et al., 2022) and population heterogeneity (Delvigne and Goffin, 2014).

Both, positive effects like increased cell viability (Enfors et al., 2001) and negative effects, such as reduced growth and production (Lara et al., 2017), have been reported in the presence of environmental gradients. In either case, the predictability of the process performance at different scales is weakened, which contributes to the complexity of the scale-up challenge.

As we need to "[live] with heterogeneities in bioreactors" (Lara et al., 2006), it is important to analyse the effects of gradients in bioprocesses on the production host to estimate the impact of scale-up effects on productivity and to take counter measurements (Fig. 1). In the 1980s, Oosterhuis introduced the concept of the scale-down procedure in order to analyse large-scale gradients in lab-scale fermenters and propose optimisations for large-scale bioprocesses (Oosterhuis, 1984). The applied lab-scale bioreactors are modified to mimic gradients by using multi-compartment reactors consisting of stirred tank reactors and plug flow reactors or by using flow-inhibiting elements (Neubauer and Junne, 2016). Each of these compartments is dedicated towards a parameter extremity (e.g. glucose overflow, oxygen depletion) and mimics the average mixing times of large-scale bioreactors.

With the application of scale-down experiments, insights can be generated on the microbial population's physiology in fluctuating environments, like substrate consumption, −omics, by-product formation and growth rates (Nadal-Rey et al., 2020), which can, for example, be interpreted as advice for strain development (Löffler et al., 2016). The classical scale-down fermenters are, however, limited: different process parameter gradients cannot be decoupled (e.g. DO and glucose concentrations or pH and osmolarity) and the frequency and amplitude of the environmental changes is strongly dependent on the tested parameter and microorganism (Haringa et al., 2018a). The chosen scale-down configuration greatly influences the results, which can lead to contradictory conclusions (Nadal-Rey et al., 2020). Furthermore, the output of scale-down experiments is predominantly population averaged data. Approaches to measure population heterogeneity during scale-down experiments exist (Delvigne et al., 2009), but are not commonly applied and cannot track individual microbes over time.

Analysing the reaction of individual microbes to gradients and dynamic environments is a promising approach for a deeper understanding and better predict the microbial population dynamics of large-scale bioreactors. One possibility is to virtually take the point-of-view of a single cell travelling through different zones in a large-scale bioreactor and record what it experiences. Such a timeline of experienced changes is called "lifeline".

This review aims to introduce the reader to microbial lifelines in bioprocesses and their analysis from an interdisciplinary point of view. After an introduction to the concept and definition of lifelines, different methods to determine lifelines are presented and their contribution to bioprocess development is discussed. Finally, an overview of current challenges of the determination and application of microbial lifelines in bioprocesses is given, as well as possible future developments to further advance this field of research.

2. Lifelines – A brief introduction

The concept of lifelines was introduced in the context of computational fluid dynamics (CFD) simulations in the pioneering work of Lapin et al. (2004). In their approach, Lagrangian particle tracking is used to follow the position of massless virtual particles, representing the biomass, over time. The observed local condition in the environment of these virtual microbes is registered as a function of time, which is called "lifeline".

To get an indication of the experienced extracellular conditions, unstructured kinetics to describe the biomass are sufficient. The largest novelty in the approach of Lapin et al. (2004), however, was the combination of lifelines with structured-segregated models: each particle can be regarded as an agent (Kaul et al., 2013) and a structured metabolic model can be used to predict the intracellular composition of each particle, and thus interaction between extracellular gradients and intracellular response. Lapin et al. (2004) demonstrated, that the autonomous glycolytic oscillation in a population of *Saccharomyces cerevisiae* is synchronised in ideally mixed reactors, but the synchronism is lost with increasingly non-ideally mixed environments.

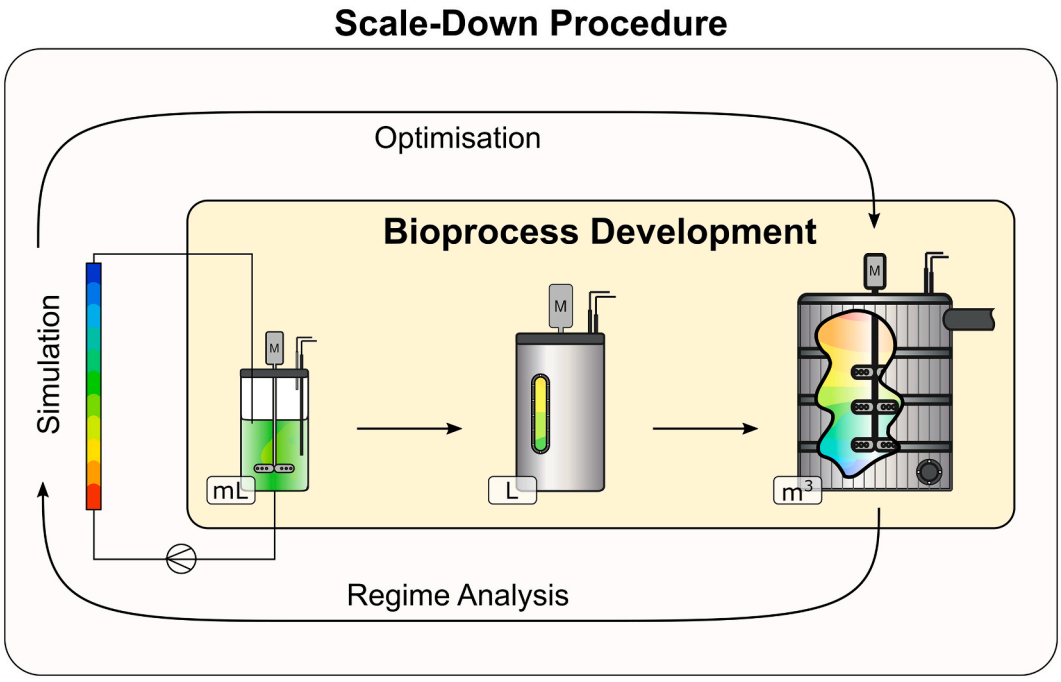

Fig. 1. Scale-up is an essential step in bioprocess development, in which the reactor volume is increased from small laboratory to large industrial fermentors. With increasing scale, more pronounced environmental gradients form. The scale-down procedure (Oosterhuis, 1984) aims to reproduce the large-scale regime in lab-scale bioreactors simulations, like multi-compartment bioreactors. Using this simulation, optimisation strategies can be tested and later be implemented in large-scale.

The next milestone in lifeline research was the corresponsding analysis proposed by Haringa et al. (2016). While an individual lifeline of a single cell represents the core of lifeline research, the true power of lifelines lies in observing the biomass as the sum of individual cells. Therefore, a statistically significant number of lifelines needs to be considered. Haringa et al. proposed three different methods to analyse a large number of lifelines statistically: regime analysis (Haringa et al., 2016), arc analysis (Haringa et al., 2016) and Fourier analysis (Haringa et al., 2018a). Using regime analysis, a lifeline is categorized based on the three metabolic regimes experienced by the microorganism, e.g. excess, limitation and starvation of a nutrient. In each regime, it is assumed that cells have a consistent metabolic response. The analysis includes the residence time in each regime and the transition frequencies from one regime to another. If the focus lies on the magnitude of the environmental fluctuation, arc analysis can be applied. An arc is the time a cell needs to cross a set baseline twice. The half-maximal substrate uptake rate can function as such a baseline. Analysing the maximum of the arc, the magnitude of a fluctuation can be estimated. Fourier analysis is the method of choice, if the lifelines are highly periodical and do not vary greatly between cells, for example in airlift-loop reactors. Of those statistical lifeline analysis methods, the regime analysis is most widely applied (Kuschel et al., 2017; Kuschel and Takors, 2020; Siebler et al., 2019).

By using lifelines for the analysis of bioprocesses from a cell's perspective, the cell itself is put into focus. As this notion spreads from computational modelling to other research fields, the need for a differentiated definition of the term "lifeline" arises.

In a bioreactor, a microbe moves rapidly on a specific trajectory (Fig. 2). Due to process parameter gradients in large-scale bioreactors, the trajectory crosses zones with different parameter conditions. From a microbe's point of view, this results in a condition-vs-time series of environmental conditions. Environmental changes, however, cause intracellular reactions, so that a cell on a trajectory in a heterogeneous environment will also experience an intracellular condition-vs-time series. Different cells will encounter different environmental changes in magnitude, duration and frequency, leading to individual environmental and intracellular cell histories and eventually population heterogeneity (Delvigne and Goffin, 2014).

Definitions of the term "lifeline" differ (Table 1). The first concept defines the trajectory itself as the lifeline, while the cell's perception and reaction are attributes of the lifeline (Kuschel et al., 2017; Kuschel and Takors, 2020; Wang et al., 2020b) or can be analysed with respect to environmental fluctuations and metabolic state (Lapin et al., 2004; Lapin et al., 2006; Siebler et al., 2019; Wang et al., 2020b). The second definition focusses on the cell's perception of their environment. The lifeline is the condition-vs-time series from a microbe's point of view (Haringa et al., 2016; Haringa et al., 2018b; Haringa et al., 2018a;

Table 1
Literature overview of lifeline definition. X indicates the definition stated in the publication, while (X) shows that the authors acknowledge a broader term for the definition of lifelines. Comments written in () point out, how the authors see the other aspects of lifelines outside their own definition. References marked with (*) did not use the term "lifeline", but applied the concept.

	A lifeline is a …		
Reference	Trajectory	Environmental condition vs. time series	Metabolic condition vs. time series
Anane et al., 2018		X	(X) ("study response kinetics")
Bisgaard et al., 2020		X	
Hajian et al., 2020		X	(X) ("readout of lifeline")
Haringa et al. 2016, 2017, 2018b, 2018a, 2022		X	(X) (lifeline readout; evaluation of metabolic response")
Ho et al., 2019, 2022	X		
* Jones et al., 2016	(X)	X	(X)
Kuschel et al., 2017	X	(X) ("experienced by microorganism")	(X) (μ-lifeline shown)
Kuschel and Takors, 2020	X	(X)	(X) (μ-lifeline shown)
Lapin et al., 2004, 2006	(X)	X	(X) (analysis of intracellular concentrations in time)
*Liu et al., 2016	(X)	X	
*Loomba et al., 2018	(X)	X	
*Marshall and Sala, 2011	X	(X)	
*McClure et al., 2016		X	
Perner-Nochta and Posten, 2007	X	(X) (light lifeline shown)	
Schmitz et al., 2019			X
Siebler et al., 2019	(X) ("flowing along lifelines")	X	(X) (translation of environmental changes to $Y_{P/X}$)
Wang et al., 2020a		(X)	X
Wang et al., 2020b	(X)	X	(X)
Zieringer et al., 2021		X	(X) (Lifelines serve as input for biological models)

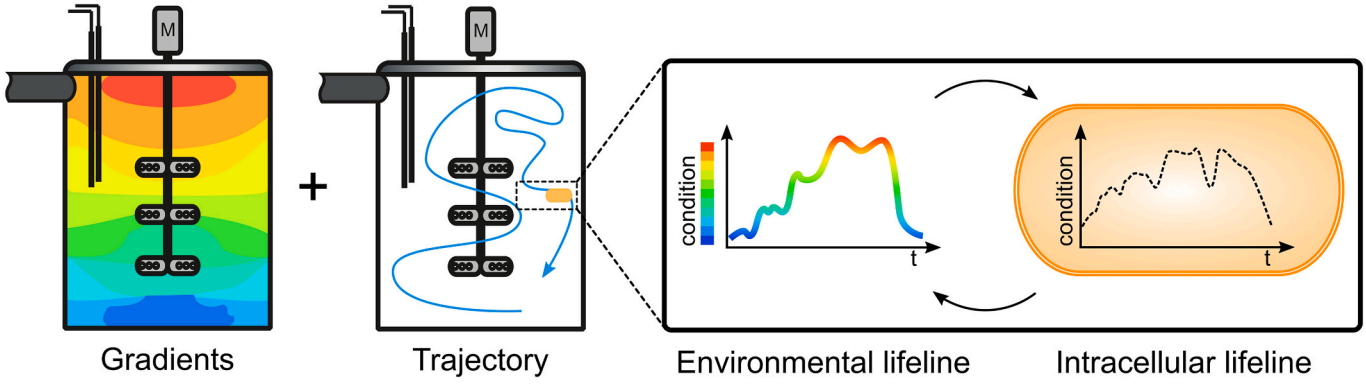

Fig. 2. Due to non-ideal mixing, environmental gradients form in large-scale bioprocesses. By following the trajectories of single cells in the bioreactor, the spatial gradients are seen as temporal fluctuations from a cell's point of view, which is called "lifeline". The environmental lifeline, which can also be described as extracellular lifeline, acts as input signal for cells and causes fluctuating reactions in the cell in the course of time, which is the intracellular lifeline.

Siebler et al., 2019). Using the lifeline, the metabolic state of a cell can be assessed (Hajian et al., 2020; Haringa et al., 2018b; Wang et al., 2020b; Wang et al., 2020a). The third concept is closely related to the second: a lifeline can also be an intracellular condition-vs-time series (Haringa et al., 2016).

Lifeline research cannot be conducted without the cell as agent to perceive the environment. It is therefore reasonable to include the cell's perception and interpretation of the gradients into the definition of lifelines. One option to account for the difference between perception and interpretation of gradients is to differentiate between environmental (extracellular) lifeline and intracellular (metabolic) lifeline. Environmental and intracellular lifeline, however, are inseparably connected, because they influence and shape each other. In some cases, environmental and intracellular lifelines cannot be clearly distinguished. An uptake-rate–vs–time series, as applied frequently in works of Haringa et al., is strongly dependent on both the environmental condition and the metabolic state of a cell.

All research on lifelines has in common, that it aims to assess the metabolic impact of large-scale gradients on microorganisms and microbial populations. This information can be valuable for insights into bioreactor performance (Siebler et al., 2019), guiding future strain engineering (Zieringer and Takors, 2018), bioreactor design and operation (Hajian et al., 2020; Kuschel et al., 2017; Lapin et al., 2004) and paving the way for efficient and successful bioprocess scale-up (Wang et al., 2020b; Wang et al., 2020a).

3. State-of-the-art of lifeline determination

Lifeline research has its roots in modelling, and as such, it is by far the most established approach to determine a microbe's trajectory and environmental and intracellular lifeline. Alternatively, or additionally, experimental approaches can be used to capture lifelines, diversifying the possibilities of acquisition and gaining knowledge (Fig. 3). In bioreactors, flow-following particles can be applied. Radioactive particles, which are dragged along the flow, are used to capture trajectories (Sabri et al., 2018), while Lagrangian sensors can detect parameter fluctuations as they are dragged along, essentially recording an environmental lifeline (Bisgaard et al., 2020). On the other side of the spectrum, precise environmental manipulations in microfluidic devices combined with live-cell imaging apply an artificial environmental lifeline on living microbes to observe intracellular lifelines (Ho et al., 2022). Traditional scale-down bioreactors mimic gradients of large-scale conditions. In a perfectly mixed single-vessel scale-down reactor, all microbes will follow the same recordable lifeline, but for multi-compartment scale-down reactors, this is not possible. For this reason, scale-down studies are excluded in the following section and the reader is referred to Nadal-Rey et al. (2020) for a review of recent scale-down experiments.

In the following sections, the possibilities to access microbial lifelines by measuring, modelling and mimicking will be regarded in more detail, highlighting the potential of every technique as well as major challenges.

3.1. Measuring

Ideally, a single-cell trajectory or environmental lifeline would be measured in situ. Process parameters like pH, shear stress and DO as well as substrate, product and by-product concentrations would be reported by a free-floating sensor the size of a microbe. Measuring process parameters in today's large-scale fermentations can include (multiple) stationary sensors or moveable lance-based sensors, as was reviewed by Nadal-Rey et al. (2020). These approaches measure coarse-grained gradients, but fail to capture trajectories or environments of individual cells. Flow-following particles offer a reasonable approach for measuring either trajectories or environmental lifelines in actual large-scale bioreactors. Conceptionally, those particles take the place of a single cell as agent in the bioreactor. They are dragged along the flow in the bioreactor because they are small (smallest radiation particle 0.1 mm, smallest sensor particle 7.9 mm) and minimally affected by buoyancy and settling, thereby being able to mimic the microbe's movement (Bisgaard et al., 2020). Particles equipped with a sensor are also known as Lagrangian sensor devices.

To measure a position-vs-time series, a particle needs to be tracked either optically or by capturing emitted radiation. Optical techniques require transparent liquids and tank walls, which is very rare in large-scale bioreactors. The application of radioactive particles can circumvent this obstacle. With radioactive particle tracking (RPT) the motion of a single γ-ray-emitting particle is recorded via several sodium iodide scintillation detectors (Bashiri et al., 2016). The particle is small (~ 1 mm (Bashiri et al., 2016)) and can be adapted in its density to fit the density of the target-phase (Sabri et al., 2018). Those attributes increase the flow-following capability. The detectors need to be placed strategically and close to the bioreactor in positions, that cover the whole bioreactor volume and circumvent the detection of γ-rays by more than one detector at a time (Bashiri et al., 2016). With computer-aided RPT, particle trajectories, local hydrodynamics, the liquid velocity field, turbulence kinetic energy and Reynolds shear stress can be measured (Sabri et al., 2018).

RPT provides only positional data. Flow-following sensors can shed light into the environment that a moving cell encounters in bioreactors. By recording the sensor parameters in a large-scale bioreactor, this coarse-grained environmental sensor-lifeline gives insight into possible environmental changes in the actual bioreactor during the large-scale fermentation. The flow-following sensor can report measurements for multiple hours (Zimmermann et al., 2013) to two weeks (Todtenberg et al., 2015), resembling the timeframe of fermentation and thus, the lifeline span of microorganisms in fermenters. A single particle can be equipped with multiple sensors for different parameters. For now, sensors measuring temperature and pressure are most common, but there are efforts to measure combinations of pH, dissolved oxygen concentration, glucose concentration, potassium concentration and conductivity (Bisgaard et al., 2020).

Sensor-based environmental lifelines are limited in their ability to depict lifelines due to challenges in their flow-following capacity and detection speed. Flow-following particles need to have a size of maximal 1 mm to adequately follow the flow in a STR (Hofmann et al., 2022), but the smallest sensor particle has a diameter of 7.9 mm (Lauterbach et al., 2019). This only allows the tracking of coarse-grained, approximated lifelines. Furthermore, the finite response time of the integrated sensors forecloses the detection of fast dynamics. For example, current DO sensors in flow-following particles have a steady state response time of 4 s (Bisgaard et al., 2020). If the sensor comes in contact with a different DO zone for less than its response time, the signal will be smoothed the shorter the contact duration is. Shorter contact with a different DO zone in a bioreactor would not be detected. Additionally, different parameters need different sensors, which have different response times.

Another substantial challenge is finding or financing large-scale bioreactors for research purposes (Nadal-Rey et al., 2020). Companies may not object to providing access to their bioreactors if the technique adds value, is easy to apply and does not compromise the bioprocess by contamination. Unfortunately, for trajectory tracking using techniques like radioactive particle tracking, detector arrays are needed in close proximity to the bioreactor (Sabri et al., 2018). The other possibility is to use radio transmission to extract the data, but the signal is lost when the distance is larger than 4 m (Lauterbach et al., 2019). This distance is decreased, when the sensor transmits from within a steel enclosure or complex media. Sensor particles are an academic playground and progress in developing a ready-to-use technology might accelerate the transfer to industry, and thus, available large-scale data.

3.2. Modelling

Modelling was the first feasible approach to capture microbial

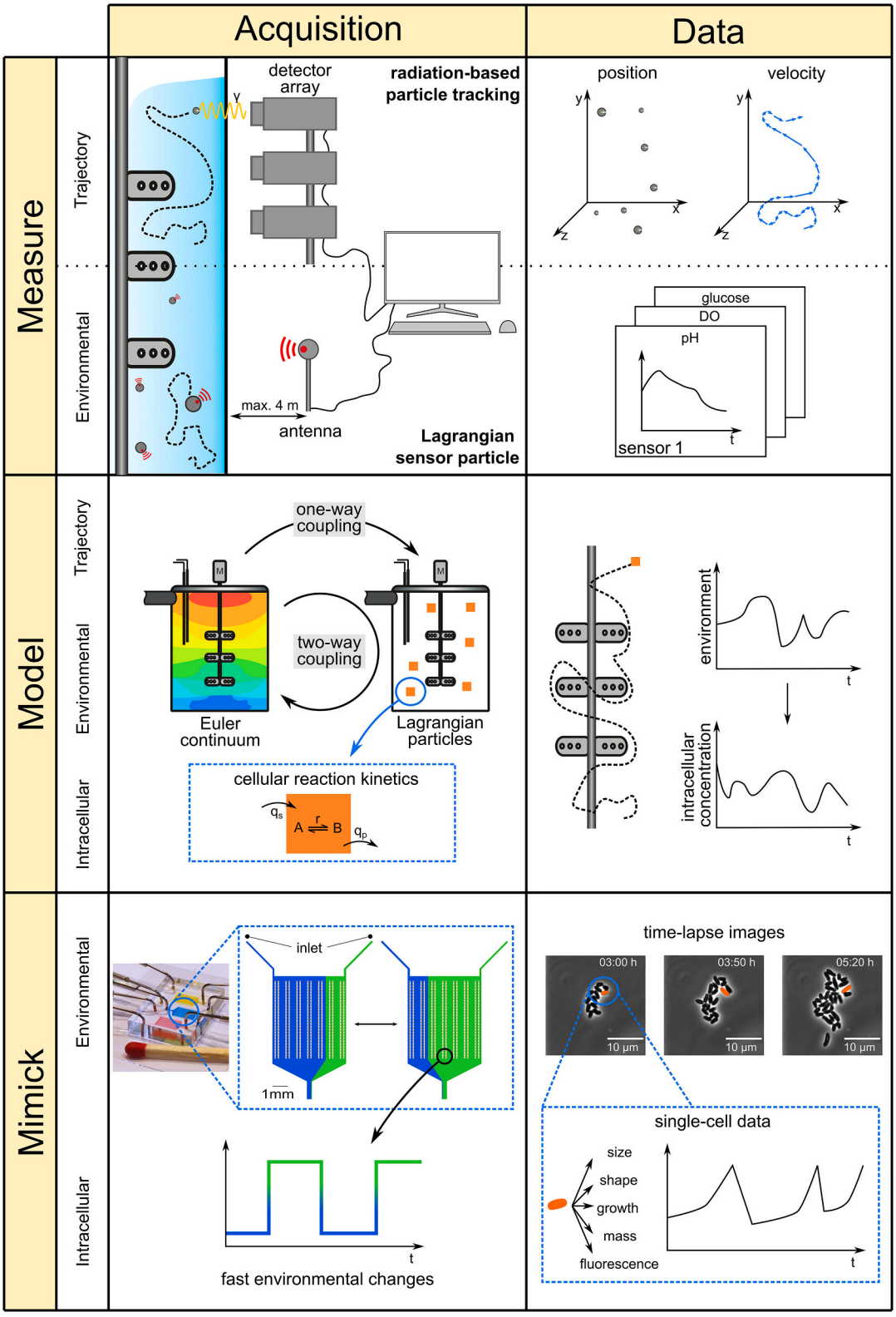

Fig. 3. Lifelines can be assessed using methods of measuring, modelling and mimicking. Trajectories and environmental lifelines can be approximated in bioreactors using flow-following particles. By tracking a γ-emitting, trajectories are recorded, which can be used to reconstruct the position and velocity field. Lagrangian sensors detect environmental changes while they are dragged along the flow and transmit the data on radio frequencies. Trajectories, however, cannot be extracted. Modelling lifelines gives computational insights into trajectories, environmental and intracellular lifelines. The model has to be adapted accordingly, choosing between one- and two-way coupling and the integration of cellular reaction and regulation kinetics. Microfluidic single-cell cultivation is a tool to investigate cellular reactions to environmental changes. Coarse-grained, artificial environmental lifelines are mimicked to observe the resulting intracellular lifeline.

lifelines. For this, a minimum of two models are needed, which interact with each other: a model for the bioreactor environment and a model that describes the cell. Most common for calculating fluid flow for gradient or lifeline modelling is the use of CFD models (Hajian et al., 2020), either using the finite volume approach (often with a frozen flowfield) (Haringa et al., 2016; Kuschel et al., 2017; Zieringer et al., 2021) or, more recently, with Lattice-Boltzmann (Haringa, 2022), but less calculation-intensive approaches gain attention like CFD-based compartment models (Haringa et al., 2022; Nadal-Rey et al., 2021).

The fluid dynamics of the bioreactor are typically modelled using an Eulerian approach wherein the flow and distribution of other relevant parameters, like gas holdup or nutrient concentration, are described by a continuum (field based) approach. In this framework, equations for fluid motion, like the Navier-Stokes equation, and the equations to calculate turbulence, mass transfer and substrate distribution can be included (Hajian et al., 2020). As substrate uptake of microbes alters the nutrient distribution, unstructured black-box substrate uptake kinetics are often applied in the Euler framework (Hajian et al., 2020), where the local nutrient specific uptake rate q_s is directly linked to the local nutrient concentration C_s.

The cell itself is modelled in a Lagrangian frame, meaning it is described by a point-particle of which the position is explicitly tracked through integration of the equations of motion. Microbes, which have a small diameter of microns, can be seen as a flow-following, massless particles; to account for turbulent motion, a random walk model is typically added to the equation of motions. This simplifies the trajectory calculation, because it may be assumed that the cells immediately adapt to the local fluid velocity, and velocity differences between the cell and surroundings do not need to be computed. For each cell, equations for substrate uptake and intracellular reactions can be included by a structured kinetic model.

By combining the Euler approach and Lagrange framework, the cell's reaction (in the Lagrangian framework) can be coupled to the environment it experiences (in the Eulerian framework), for example a change in substrate uptake rate as the cell travels through a region with different substrate concentrations. To decrease calculation time, the Euler-part of the model is often frozen in time, so that the cells travel through pseudo-stationary gradients. It is assumed, that the cells metabolism will not influence the overall gradient in the limited time (a few hundred seconds) that can be calculated by Euler-Lagrange simulation in a reasonable time. If the Euler-environment influences the Lagrange-cell, but is not changed by the cell's reaction, it is called one-way coupling. Yet, it is more realistic that cells will shape their environment over time. This method of two-way coupling has, however, seldom been used (Lapin et al., 2004; Lapin et al., 2006). For a more detailed view on how to set up lifeline models using CFD, the reader is referred to Hajian et al. (2020).

Typically, a large number of cells ($\sim 10^5$) is needed in Euler-Lagrange simulation, to account for an even biomass distribution and therefore prevent artificial variations in the reaction distributions (Haringa et al., 2017b). Each simulated cell represents multiple cells, that have the same trajectory, to incorporate the total biomass concentration into the model (Haringa et al., 2017b). Even more cells need to be simulated for models using structured kinetics, which considers differences in individual cells' reactions, because variations in too few particles may otherwise lead to artificial fluctuations in the intracellular state. A large cell number demands a lot of calculation time. Therefore, priorisation is needed on the most important reactions for the target research question and how much they can be simplified to still be representative (Tang et al., 2017). Tang et al. (2017) introduced the "9-pool-model", reducing the penicillin production in *Penicillium chrysogenum* to five metabolite pools and four enzyme pools, which resemble the capacity of a metabolic pathway. These pools were connected by 10 reactions. Haringa et al. (2018b) later applied this model in lifeline modelling. The 9-pool model focuses on metabolites and enzymes, but ignores regulatory mechanisms concerning transcription and translation, which can delay a cells reaction to the environment. Zieringer et al. (2021) demonstrated a model, that accounted for non-instantaneous cellular reaction to environments.

Experimental knowledge about the bioreactor geometry and operation details as well as microbial kinetics is needed to establish and validate sophisticated models. The bioreactor geometry includes the height-diameter ratio, the number, position and geometry of turbines, ideally including according experimental data concerning velocity, kinetic energy and energy dissipation (Haringa, 2022), as well as the point of addition for any added substance like substrate, pH or gases (Haringa et al., 2016; Kuschel et al., 2017). To model the operation of the bioreactor, stirring speed, broth density, broth rheology, aeration and feeding rate need to be known. The power input predicted by CFD is a parameter that is commonly used for verification with experimental large scale data. Furthermore, the mixing time to achieve 95% homogeneity is also a parameter of interest for verification of model predictions. In large-scale bioreactors, the mixing time can be estimated using the circulation time, which in turn can be experimentally determined by measuring the lag time between top-fed addition of a pH active substance and the response of a bottom-stationed pH sensor (Haringa et al., 2016). To integrate the biological model, the microbe specific uptake rate q_s of a substrate is the required minimum (Haringa et al., 2016; Kuschel et al., 2017; Kuschel and Takors, 2020; Zieringer et al., 2021). Often, q_S is calculated from experimentally determined biomass specific yield coefficients $Y_{X/S}$ and the specific growth rate μ (Kuschel et al., 2017). For the substrate uptake, the substrate specific uptake constant K_S also needs to be experimentally determined. The further the focus of lifeline modelling shifts to intracellular lifelines, the more experimental data concerning the microbial dynamics is needed. As example, Zieringer et al. (2021) regarded the intracellular ATP demand of a cell travelling through the bioreactor. For this, they integrated experimental data concerning the transcriptional response to repeated starvations pulses, ATP consumption growth-independent maintenance, the total mRNA content, as well as the RNA-polymerase transcription velocity, the translation elongation rate and RNA and protein degradation rates.

Lifeline modelling provides access to both environmental and intracellular lifelines with high temporal resolution. Models can more easily be fitted to the research question than actual large-scale bioreactors or other wet-lab experiments by adding relevant equations to the model framework or changing input parameters. This is what makes lifeline models very useful in optimizing bioprocesses. However, modelled lifelines typically consider a very narrow timeframe in the mid to late fed-batch phase of a bioprocess (Haringa et al., 2016) and also exclude cell division to simplify the model. Refining the model to better fit the research question, means to add more equations, which increases the computational power needed. To calculate lifelines for 10^5 cell with a duration of a few hundred seconds, the calculation time on a high end desktop computer can easily last weeks (Haringa et al., 2018b). Most lifeline models regard one process parameter (most often glucose availability) in a monophasic environment. However, there are first developments to examine two process parameter at once (Kuschel and Takors, 2020) and to take aeration bubbles into consideration (Siebler et al., 2019). Adapting models to less frequently used organisms is also demanding, as knowledge about reaction kinetics and regulatory networks in these organisms is limited. Often, assumptions from model organisms are applied in these cases, which limits the model to unstructured kinetics (Siebler et al., 2019).

3.3. Mimicking

The application of microfluidic single-cell cultivation and analysis gains momentum in biotechnological research and bioprocess development (Dusny and Grünberger, 2020; Ortseifen et al., 2020). Using microfluidic single-cell cultivation as a tool to mimic lifelines has two major advantages compared to modern scale-down approaches (Haringa et al., 2018a), which was recently demonstrated for the first time (Ho

et al., 2022; Täuber et al., 2022). Microfluidic systems have precise environmental control which is decoupled from microbial bioanalytical activities and individual cells can be tracked over time with the help of live-cell imaging to estimate the influence of cell history effects.

In microfluidics, fluids are precisely manipulated in structures on the micrometer scale. This allows high experimental parallelisation at little resource usage. Microfluidic chips applied in live-cell imaging commonly consist of the polymer PDMS (polydimethylsiloxane) (Fig. 3 - Mimicking) due to its optical properties, biocompatibility, oxygen permeability and easy replication by moulding. In these chips, microfluidic structures like monolayer growth chambers, mother machines or single-cell traps can be applied for the hydrodynamic trapping of cells (Grünberger et al., 2014). The trapping structures can be aligned in arrays, which are positioned in parallel to the laminar flow. For different microorganisms or research questions, a diversity of other trapping methods is available (Johann, 2006; Nguyen et al., 2021a).

The fast mass transport in microfluidic devices provides the necessary environmental control for mimicking environmental lifelines. The microfluidic channels are constantly perfused, replenishing substrates, removing metabolites. Thus, the environment is constant and precisely applicable in flow channels. Cells in structure-based trapping regions are supplied via diffusion from the supply channel, which is very fast in micrometer scale (Ho et al., 2019). The environmental control increases with decreasing cultivation volume of the trap, because diffusion time decreases with the distance and fewer cells alter the environment (Grünberger et al., 2014). Changing the medium in the supply channel will change the medium in the trapping regions accordingly, effectively decoupling the environmental change from the microbe's metabolism.

The medium in the channels has to be exchanged very fast for precise correlation between environmental change and the observed reaction. Technically, dynamic medium exchanges in the range of seconds can best be mediated using pneumatic on-chip-valves or external pressure pumps (Täuber et al., 2020b). Based on this research, a simple microfluidic set-up for dynamic microfluidic single-cell cultivation (dMSCC) was established, which uses pressure driven pumps to shift the laminar boundary layer (Fig. 3), thereby exchanging the medium in the switching region (Täuber et al., 2020a). Using dMSCC, complete medium exchange in the monolayer growth chambers can be ensured with medium oscillations down to 5 s (Täuber et al., 2020a). Only Nguyen et al. (2021a) reported faster fluctuations of 3 s. Their system combines a dial-a-wave-junction (Ferry et al., 2011), which enables fast medium exchange in the inlet channels, and chemical trapping of microbes with poly-L-lysine in the channels, so that mass transport is convective (Nguyen et al., 2021a). Fluctuation duration, frequency and amplitude can easily be modified to fit the research question and can be programmed to seemingly arbitrary patterns. These fluctuation profiles can be inspired by modelled lifelines, therefore applying coarse-grained environmental lifelines to microbes (Ho et al., 2022).

Detection of the resulting intracellular lifelines needs to be non- (or minimally) -invasive to ensure the individual microbe's vitality for further tracking in time. Microscopy, especially live-cell imaging, is the most essential technique for microfluidic live-cell imaging, which can yield data on cell count, morphometrics like cell length and area, as well as cell mass (Dusny, 2020). With quantitative phase microscopy and the exploitation of the cell's refractive index, even the biomass of a single cell can be estimated (Popescu et al., 2008). Fluorescent chemicals, metabolites and proteins are another popular choice to detect cellular reaction to environmental changes. The fluorescent output signal should be quantifiable and sufficiently fast to react to changes in the timeframe of environmental lifelines. The autofluorescence of intracellular metabolites like NADPH can function as such an output signal (Zhang et al., 2018). Furthermore, a wide palette of metabolite-responsive transcription factor-based biosensors has been developed (Monteiro et al., 2019). The metabolite of interest acts as ligand for the transcription factor, which leads to the transcription of the reporter gene. In this way, the intracellular concentration of products (Binder et al., 2012) and metabolites acting as indicator for the flux through a pathway (Monteiro et al., 2019) can be monitored. A drawback of the application of translation dependent biosensors is, that the lag-time between sensing and expression needs to be considered as well as protein degradation dynamics to also decrease the output signal in an appropriate timeframe (Dusny, 2020). Alternatively, fluorescent protein biosensors that change their intensity (intensiometric) or the excitation or emission spectrum (ratiometric) based on the concentration of a target can react to changes in real-time (Bolbat and Schultz, 2017).

Mimicking bioprocess relevant lifelines in microfluidic devices is still in it's infancy, but it promises to make live-cell intracellular lifelines accessible. Furthermore, the high parallelisation of microfluidic cultivations enables the recording of a statistically significant number of lifelines. As all cells experience the same environmental lifelines, effects of intrinsic noise on intracellular lifelines can be studied.

4. Application of lifelines in bioprocess development

The development of new bioprocesses is an endeavour, that has the risk of failing in the late stages. To limit the risks, modern bioprocess development keeps the end in mind and retro-engineers the process (Noorman and Heijnen, 2017). It is important to plan the large-scale process first before starting process development, so that possible problems can be tackled before scale-up or circumvented by choosing a different approach. Scale-down procedures are an important tool when designing bioprocesses with the end in mind (Straathof et al., 2019) and lifeline simulation and analysis can develop into a valuable extension (Fig. 4).

The analysis of environmental lifelines already helps to guide classical scale-down experiments. One major challenge is to adequately design the scale-down set-up to closely resemble the large-scale gradient (Haringa et al., 2017a). Haringa et al. (2018a) demonstrated how either of regime, arc and Fourier analysis of lifelines can be used to develop scale-down reactor concepts. Taking regime analysis as an example, the number of regimes resembles the number of scale-down compartments for each regime, while the volume of each compartment and the flow between the compartments is based on the regime volume fraction and the regime residence time distribution (Haringa et al., 2018a). An approach to guide a non-traditional scale-down was shown by Ho et al. (2022), who discretised computational lifelines into a starvation and excess regime and directly applied the regime profile to bacteria in a microfluidic set up. Today, using the lifeline knowledge to guide scale down is the most common application for lifelines in bioprocess development (see Table 2).

With the analysis of environmental lifelines, it is also possible to test (Haringa et al., 2018b) and compare (Liu et al., 2016) different bioreactor configuration to optimise the large-scale set-up (Fig. 4). An altered feed addition location can, for example, substantially decrease the productivity loss, as a more equal substrate distribution leads to lower stress amplitudes and consequently less inhibition of production (Haringa et al., 2018b). CFD modelling without Lagrangian particles suffices to estimate the magnitude of gradients and change the reactor design accordingly. Still, questions of the relevance of a gradient magnitude for a bioprocess is best investigated with the durations and frequencies observed in a computational microbe's lifeline.

The simulation of intracellular lifelines as a reaction to large-scale heterogeneity facilitates the identification of metabolic bottlenecks and limitation of strains in fluctuating environments and suggests strain engineering targets. As such, genetic alteration to decrease the ATP demand for transcriptional adaption to changing environments (Siebler et al., 2019) or other cellular stress programs (Ziegler et al., 2021) were suggested. Another target derived from lifeline analysis is to increase K_s for less susceptibility to substrate fluctuations (Haringa et al., 2018b).

While using lifelines for guiding representative scale-down already finds application (Table 2), suggestions to bioprocess optimization and strain engineering remain vague. For now, new strain engineering

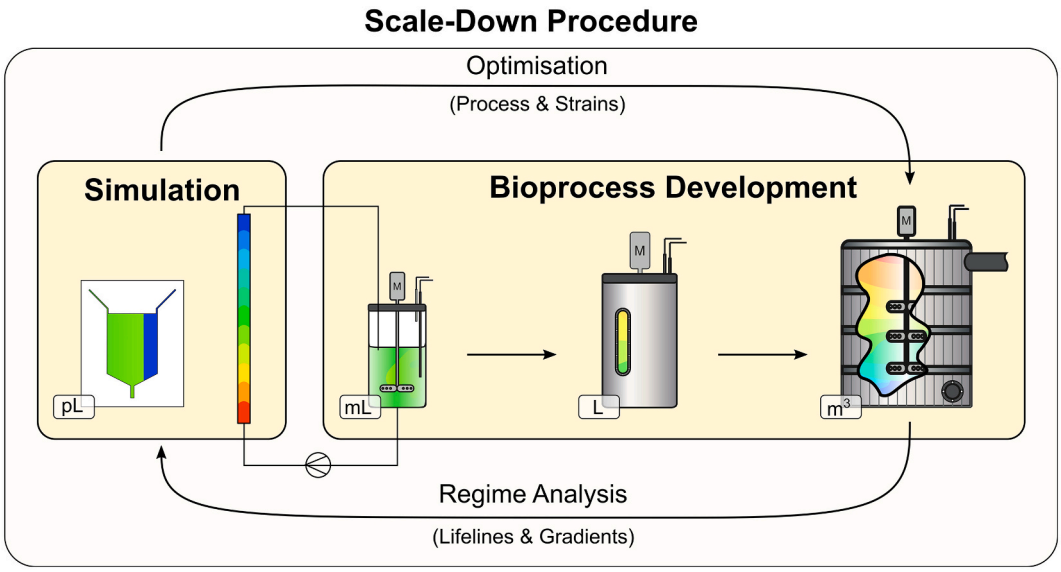

Fig. 4. The application of lifelines in bioprocess optimisation adds new tools to the existing scale down procedure of Oosterhuis (1984). In traditional scale down, the regime is analysed with regard to gradients, which are mimicked in scale-down bioreactors. The resulting knowledge is applied to optimise the bioprocess. Using the lifeline procedure, the regime and its effect on the cell's metabolism is analysed from the cell's perspective as lifeline. Analysis of the lifelines can be applied to guide scale down experiments or lifeline mimicking, but it can also directly propose bioreactor design and strain optimisation.

targets are derived from scale-down studies like Löffler et al. (2016), which are also used to build more sophisticated models (Siebler et al., 2019; Zieringer et al., 2021). The possible targets named by Zieringer et al. (2021) were engineered by Ziegler et al. (2021), without referring to the role of lifeline analysis in their research. Most recently, Minden et al. (2022) demonstrated how lifeline analysis can inspire wet-lab research that contributes to better models and provides a basis for engineering metabolic targets. All in all, lifelines can help to guide strain optimisation and bioreactor design and operation by providing a glimpse into large-scale conditions prior to scale-up, but their contribution today is limited to refining scale-down bioreactors. More linked, interdisciplinary research can advance the role and application of lifeline research in bioprocess development.

5. Future directions

The research on lifelines in bioreactors has greatly developed in the last decade. With a coherent statistical analysis of lifelines at hand (Haringa et al., 2016), applications of lifelines became accessible for scale-down purposes and lifeline research entered its second phase. The next phase is now emerging with the concept of lifelines slowly emancipating from modelling to include interdisciplinary approaches like lifeline measuring and mimicking (Fig. 5). Still, these techniques need further development and integration to determine and use representative lifelines.

Flow-following particles can provide insights into actual, albeit coarse-grained, large-scale bioreactor trajectories and environmental lifelines. In the future, measuring environmental lifelines using Lagrangian sensors has to find the balance between the trade-offs of a lifeline's complexity and accuracy. On the one side, the duration and complexity of the measured lifelines should be increased. This includes the combination of more sensors into the particle sphere, to measure more parameters in parallel (Lauterbach et al., 2019). There, one major challenge is to achieve the synchrony of the different sensor signals. Optimally, the measurement should also last for the whole fermentation process, which can be achieved by improving the sensor's energy efficiency and the battery capacity (Lauterbach et al., 2019). Both those improvements would greatly increase the achievable complexity, but they are likely to lead to an increase in the sensor's size. Increasing the sensor's size reduces the flow-following capability, decreasing the representability of the measured lifelines. The flow-following capability of Lagrangian sensors is especially limited at high impeller speeds (Bisgaard et al., 2021a), and high aeration rates, because the sensor is not adapted to the density of the disperse fluid.

It is still very difficult to obtain data from industrial scale bioprocesses for public research, from which the whole lifeline community would greatly benefit. To make industrial partners with large-scale bioreactors more inclined to apply flow-following sensors in their reactors, the recorded lifeline data could be stored locally in the particle (Bisgaard et al., 2020) rather than transmitted in real-time, to be exported after the fermentation. This would erase the need for closely located and expensive transmission receivers or other structural changes in the production facility. This would suffice for lifeline research questions, but not for the application of sensor particles for operating purposes. Another downside of this approach is the further increase of the sensor's weight and size, influencing the flow-following capability.

Measured lifelines are either trajectories or environmental lifelines. The generated data can be used to provide input for models and validate them (Bisgaard et al., 2021b; Hofmann et al., 2022). First commercial applications of measured lifelines from sensor particles are offered to advise on operation condition and bioreactor design (Freesense ApS, 2022). Furthermore, the environmental lifelines gained from measuring in reactors can directly be mimicked using microfluidic single-cell cultivation, to access the according intracellular lifeline.

The modelling of lifelines strives to enhance the realism of both the environmental and the intracellular lifeline. Many improvements have been made in recent years that enhanced the depiction of the abiotic phase in bioprocesses. It is now possible to simulate environmental lifelines of more than one parameter at a time (Kuschel and Takors, 2020) and include gas bubbles as a third phase for the environmental calculation (Siebler et al., 2019). Still, other aspects of the abiotic phase have yet to be integrated into lifeline modelling. For now, the vast majority of lifeline simulations depict a timeframe of a few hundreds of seconds during the late fed-batch phase in a bioprocess, while realistic lifelines should be traceable throughout the fermentation. Therefore, models of the abiotic phase need to account for changing broth volumes in time. Nadal-Rey et al. (2021) demonstrated a dynamic compartment model that calculated gradients and their development during the whole fermentation process. Furthermore, the bioreactor environment is also influenced by the organism's reaction to it. More accurate environments

Table 2
Overview of studies involving lifelines in literature. Each study is introduced with the study setup, the study's conclusion (C), the type of lifeline (L) that has been analysed, as well as the key contribution to the field of lifeline research (KC). For each reference, the lifeline research was categorized into what was done with the lifelines (recorded, analysed, used to guide scale down experiments) and what the author sees for applications of the insights generated with lifelines for the future, e.g. support bioreactor design or strain engineering.

Lifeline study **C**: conclusion **L**: Type of lifeline **KC**: Key contribution to lifeline research	Record	Analyse	Guide Scale-Down	Propose Bioreactor Optimisation	Propose Strain Engineering	Reference
Saccharomyces cerevisiae, glucose gradient, 68 L STR, Euler-Lagrange **C:** Increased environmental heterogeneity caused desynchronization of glycolytic oscillation **L:** Intracellular lifeline: intracellular NADH concentration vs. time as result of internal glycolytic oscillations and external heterogeneity. **KC**: first modelled lifelines	Not shown	x				(Lapin et al., 2004)
Escherichia coli, glucose gradient, 900 L STR, Euler-Lagrange **C:** Different scales lead to differences in cell viability **L:** Intracellular lifeline: PEP/PYR ratio dependent on glucose concentration **KC**: Application of lifelines to more bioprocess relevant research question	Not shown	x				(Lapin et al., 2006)
Carthamus tinctorius L., shear stress, 5 L and 15 L STR, Euler-Lagrange **C:** The product of a lifeline's maximal shear stress and its frequency improves the accuracy of models for shear-dependent death kinetics. **L:** Environmental lifeline: shear stress vs. time series **KC:** Lifelines were used to derive a new parameter that can facilitate the shear stress comparison between bioreactor setups and operations during scale-up.	x	x		x		(Liu et al., 2016)
Penicillium chrysogenum, glucose gradients, 54 m^3 STR Euler-Lagrange **C:** Statistical analysis of lifelines can provide an approach to design representative scale-down experiments **L:** Environmental uptake lifelines: q_s vs. time series **KC:** Introduction of regime and arc analysis for lifelines	x	**x**	x			(Haringa et al., 2016)
Pseudomonas putida KT2440, glucose gradients, 54 m^3 STR, Euler-Lagrange, one-way coupled cell cycle model **C:** Subpopulations with different growth phenotypes form in a heterogeneous bioreactor as a consequence of diverse microbial lifelines. **L:** Intracellular: growth rate, ATP consumption rate and C-phase duration vs. time series **KC:** Improved understanding of population heterogeneity generation in large scale bioreactors.	x	x				(Kuschel et al., 2017)
Saccharomyces cerevisiae, glucose gradients, 22 m^3 STR, Euler-Lagrange **C:** Previous scale-down designs often do not resemble large-scale conditions, but this can be improved by applying lifeline knowledge to experimental scale-down design. **L:** Environmental uptake lifeline: $q_s/q_{s,max}$ vs. time **KC:** Demonstration of representative scale-down design based on statistical analysis of microbial lifelines.	x	x	**x**			(Haringa et al., 2017a)
Penicillium chrysogenum, glucose gradients, 54 m^3 STR, Euler-Lagrange CFD coupled with 9-pool metabolic model **C:** Scale-up causes a drop in pencillin production rate, which was reduced by optimizing the bioreactor design. **L:** Environmental uptake lifeline $q_s/q_{s,max}$ and intracellular lifeline of μ and metabolite pools **KC:** Showcasing the versatility of lifeline models in multiple process optimization steps	x	x	x	x	x	(Haringa et al., 2018b)
Clostridium ljungdahlii DMS 13528, CO gradient, 125 m^3 bubble column, Euler-Euler-Lagrange including metabolic models **C:** CO is severely limited in this case study and will negatively impact the assumed cell model. **L:** Environmental CO vs. time series, translated to intracellular $Y_{P/X}$ vs. time **KC:** Application of a multiphase Euler-Euler model to integrate the gaseous phase into lifeline modelling	x	x			x	(Siebler et al., 2019)
Corynebacterium glutamicum, oxygen and glucose gradients, 300 L STR, Euler-Euler-Lagrange **C:** The modelling mesh can be coarse-grained and still yield very comparable residence times of particles in the biological relevant regimes. **L:** Environmental lifeline (oxygen and glucose vs. time) and resulting intracellular lifeline (μ vs. time) **KC:** First example of environmental multiparameter lifeline	x	x	x			(Kuschel and Takors, 2020)
Escherichia coli K12 W3110, glucose gradients, 54 m^3 STR, Euler-Lagrange, cellular regulation model **C:** Microbial reaction to environmental changes propagate through the bioreactor and will not happen in the near vicinity of the environmental change.	x	x			x	(Zieringer et al., 2021)

(*continued on next page*)

Table 2 (*continued*)

Lifeline study **C:** conclusion **L:** Type of lifeline **KC:** Key contribution to lifeline research	Record	Analyse	Guide Scale-Down	Propose Bioreactor Optimisation	Propose Strain Engineering	Reference
L: Intracellular lifeline (protein, RNA, ATP vs. time) **KC:** The first coupled cellular regulation model that accounted for transcriptional and translational reaction time in the metabolic response to environmental changes. This study is also a very good example of how scale-down can provide data for modelling.						
Penicillium chrysogenum, glucose gradient, 54 m^3 STR, LB-LES - (Lattice-Boltzmann Large Eddy Simulation) – Lagrange, 9-pool metabolic model **C:** LB-LES is suitable to model lifelines **L:** Glucose uptake lifeline ($q_s/q_{s,max}$ vs. time) **KC:** By applying LB-LES instead of Euler for the continuous phase, the computational burden of lifeline modelling is drastically reduced, making longer lifelines possible.	x	x				(Haringa, 2022)
Corynebacterium glutamicum ATCC 13032, glucose gradients, 300 L STR, Euler-Euler-Lagrange, microfluidic mimicking **C:** The average perceived glucose concentration along the lifeline had a stronger influence on the growth rate than duration and frequency of starvation stress. **L:** Environmental glucose lifeline **KC:** first interdisciplinary approach to lifelines by showing how lifeline regime analysis can be used to design microfluidic mimicking experiments	x	x	Microfluidic mimicking			(Ho et al., 2022)
Saccharomyces cerevisiae CENP.PK 113-7D, glucose perturbation inspired by 22 m^3 STR, chemostat with 2 min starvation pulse **C:** Industrially relevant glucose perturbations change the energy and redox homeostasis and increase the overall metabolic maintenance demand by 2.7%. **L:** Environmental glucose lifeline **KC:** First wet-lab experiment inspired by lifelines, that has the aim of providing relevant data to refine lifeline modelling.	x	x	Pulse feed based on lifeline			(Minden et al., 2022)

can be reached with two-way coupling between environment and cell (Pigou and Morchain, 2015). While the feasibility of two-way coupling has been shown (Lapin et al., 2004; Lapin et al., 2006), only few studies apply it (Haringa et al., 2018b; Haringa, 2022; Haringa et al., 2022). In the course of a fermentation, other physico-chemical parameters will also change, for example the broth viscosity. There are first CFD-based models, that incorporate viscosity changes in the course of fermentation (Bach et al., 2017), but this technique has yet to be applied in lifeline modelling and needs further improvements concerning turbulence and rheology interactions.

On the other side of model enhancement, there is the on-going development of better biomass description. Firstly, with increasing the lifeline timeframe to a realistic fermentation length, the increase of biomass can no longer be ignored. This includes single-cell growth and division events, making it possible to shed light into cell history and ancestry effects. The first steps towards depicting population heterogeneity in growth in lifeline research have been made by Kuschel et al. (2017), who demonstrated how glucose gradients led to heterogeneity in growth rates of single *P. putida* cells, based on a cell cycle model. This showcases, how extrinsic noise leads to heterogeneity in populations, but intrinsic noise as cause for heterogeneity has not been accounted for in lifelines. Recent reviews and research papers highlight ongoing development towards more realistic metabolic models (Wang et al., 2020b) and the integration of cellular regulation into metabolic models (Zieringer et al., 2021).

Increasing the timeframe of simulations, refining intracellular models and using two-way coupling between the environment and the cells demands a very high computational cost (Haringa, 2022). In modelling, the balance between model simplifications and accurate depiction of the bioreactor needs to be found. There are multiple approaches to decrease the computational effort while keeping acceptable model accuracy. For CFD-modelling, the mesh size, which depicts the resolution of the bioreactor, can be decreased and still yield representative results (Kuschel and Takors, 2020). Another possibility is to use compartment models to describe the environment and stochastic parcel tracking to replace the classic trajectories, which requires significantly less calculation time (Delafosse et al., 2015; Haringa et al., 2022). Alternatively, the environment can be simulated using the Lattice-Boltzman approach while tracking single-cells in the Lagrangian frame (Haringa, 2022).

From a modelling perspective, the future application of lifelines research lies in predicting the process performance (Siebler et al., 2019). With lifelines, digital twins of the bioreactor (macro-twin) and the cell (micro-twin) can be established (Hajian et al., 2020). Digital twins can help in the scale-up process by reducing the need for costly pilot runs and facilitating the optimisation procedure or they could be run in parallel to the large-scale fermentation to predict performance in real time (Hajian et al., 2020; Neubauer et al., 2021).

Mimicking lifelines in microfluidic devices has the great advantage, that the change in environment is decoupled from the cell's metabolism and that the environment is very precise and defined. The quality of intracellular lifeline data generated from mimicking is very dependent on the quality of the employed environmental lifeline, both conceptionally (CFD-modelling) and practically (microfluidic implementation). Until now, research towards microfluidic lifelines has been limited to systematic oscillation measurements (Täuber et al., 2022) and the mimicking of single-parameter lifelines with two discrete parameter values (Ho et al., 2022). Actual large-scale lifelines, however, consist of multiple parameters, that change in their amplitude and frequency during the course of fermentation. Microfluidic devices have to develop towards these standards.

A first step is to broaden the spectrum of applicable and relevant parameters (Leygeber et al., 2019). Nutrient oscillations have been applied (Ho et al., 2022; Täuber et al., 2020a) and pH fluctuations can be implemented (Täuber et al., 2022). Fast and dynamic switching of dissolved oxygen and dissolved carbon dioxide, for example, have not yet

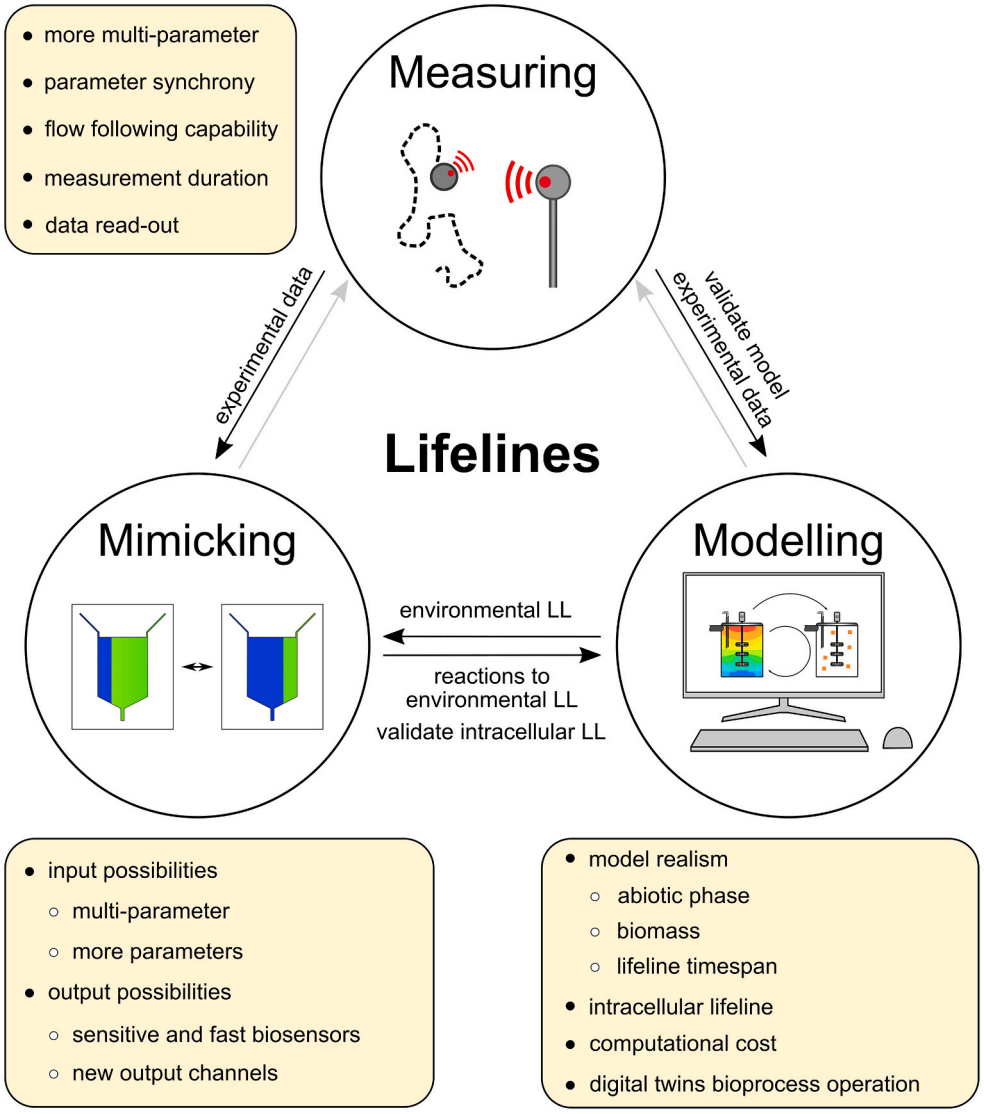

Fig. 5. Future direction, challenges and interdisciplinary cooperation of measuring, mimicking and modelling of lifelines (LL).

been shown. This would require the use of more sophisticated set-ups or other materials for chip fabrication, because PDMS is permeable for gases. In case of dissolved oxygen oscillation, the application of an oxygen scavenging thiol-ene based polymer (Sticker et al., 2020) or non-permeable materials like glass can be possible alternatives.

The combination of different parameters on-chip is another important development step. For example, it was shown in scale-down bioreactors that oxygen oscillations increase the susceptibility of *C. glutamicum* towards pH perturbations (Limberg et al., 2017). A first step to combining multiple parameters is to increase the number of possible media reservoirs and switch between those on chip by using multiple inlets (Täuber et al., 2022) or pneumatic valves. A similar technique can be applied to vary the amplitude of environmental change during the experiment. However, the proposed technique offers only stepwise changes in parameters, while gradual changes cannot be achieved in that system. Microfluidic devices that switch the environment via a dial-a-wave junction (Kaiser et al., 2018) can change seamlessly between any ratio of two environments, but those have never been applied in rapid environmental changes (Täuber et al., 2020b).

Today's limit of temporal resolution of environmental changes in microfluidic devices is 3–5 s (Nguyen et al., 2021a; Täuber et al., 2020a). An open question in lifeline mimicking is, which temporal resolution is actually necessary to capture representative lifelines. Similar to CFD-guided traditional scale-down, modelling lifelines can guide mimicking experiments in this instance. In case that faster environmental changes are required, smaller microfluidic trapping regions with fewer cells, like mother machines (Wang et al., 2010), or such structures that rely on convective and not diffusive mass transport can be used. Alternatively, variants of the picolitre bioreactors (Grünberger et al., 2012) in combination with dMSCC can be applied for this aim.

Besides the environmental input optimisation, fast and quantifiable output options need to be applied to record intracellular lifelines appropriately. Well-chosen fluorescent biosensors are a wide field that is continuously developed. Beside the microscopic and fluorescent readout, Raman spectroscopy might offer a non-invasive analysis tool for microfluidic cultivations (Chrimes et al., 2013).

Interdisciplinary research between modelling and microfluidics can be a very fruitful endeavour, that is gaining more and more attention (Bjork and Joensson, 2019; Grünberger et al., 2014; Haringa et al., 2018a). Both modelling and mimicking can benefit from each other.

Modelling not only provides environmental lifelines to mimic (Ho et al., 2022), but it can also help to design microfluidic experiments based on lifelines. Using a variation of the rational scale-down based on lifelines (Haringa et al., 2017), microfluidic experiments could be designed to test representative environmental oscillations (Fig. 4). Due to the small size of microfluidic devices, high parallelisation is possible

and can be applied for screening organisms for large-scale robustness during the early stages of strain development. This would contribute to modern bioprocess development, which starts with the end in mind (Noorman and Heijnen, 2017).

Mimicking, on the other hand, can help to validate intracellular lifeline models. By replicating environmental lifelines, the observed intracellular lifeline can be compared to the lifelines calculated by the model.

Furthermore, there is a demand in the modelling community for kinetic data based on dynamic environments that could be provided by microfluidic experiments. Observing and understanding the response of biological systems to perturbations is needed to further advance models towards digital twins (Hajian et al., 2020; Wang et al., 2018; Zieringer et al., 2021). This endeavour can greatly benefit from time-resolved single-cell data. Other valuable data for modelling can be investigated using microfluidics, like substrate uptake kinetics (Lindemann et al., 2019; Smaluch et al., 2022), flux (Monteiro et al., 2019) or gene network dynamics (Bennett and Hasty, 2009). With the application of microfluidic tools for research on population heterogeneity, a differentiation between effects of environmental and intrinsic noise is possible, which can provide data for enhancing models on population heterogeneity (Delvigne et al., 2017; Theron et al., 2018).

6. Conclusion

Lifeline analysis is an emerging field of research that aims to estimate effects of environmental heterogeneity on microbes in bioprocesses. By taking the cell's point of view in a bioprocess, single-cell data is collected. This can reveal effects which are lost in population-averaged data of bulk measurements. Lifelines can be defined as time series in either position, environment, intracellular concentration or a combination thereof. The first lifelines were simulated using an Euler-Lagrange framework, which already finds application in bioprocess development by guiding rational scale-down experiments and proposing targets for bioreactor and strain optimisation. Measuring coarse-grained environmental lifelines with flow-following sensor particles or mimicking lifeline in microfluidic devices to measure the cell's reaction are the start of new interdisciplinary approaches to lifelines. For now, mostly single-parameter lifelines are investigated for reasons of simplicity. This is in contrast to the actual lifeline experienced by a microbe in a bioprocess, which includes the entirety of all environmental and metabolic fluctuations across all thinkable scales and generations until a cell dies without having reproduced. While this lifeline will never be reached with any experimental approach, progress is still made towards establishing more bioprocess relevant parameters, parameter combination and analysis techniques for lifelines, because striving to a more accurate physical description of cells and their environments in large-scale bioreactors is still a promising endeavour. Insights on which environmental conditions should be aimed or avoided in large-scale bioreactors can lead to actionable insights on process design and operation. Another question that remains is, how closely the determined lifelines need to resemble actual microbial lifelines to reach this aim.

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CRediT authorship contribution statement

Luisa Blöbaum: Conceptualization, Investigation, Writing – original draft, Visualization. **Cees Haringa:** Conceptualization, Writing – review & editing. **Alexander Grünberger:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

We, the authors, declare no conflict of interest.

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