Chapter 1

Introduction

> ”What an organism feeds upon is negative entropy. Thus the device by which an organism maintains itself... consists in continually sucking orderliness from its environment.”
>
> – Erwin Schrödinger, *What is Life?* (1944)

This quote by Erwin Schrödinger captures a central conceptual challenge underlying modern bioprocess engineering: living cells rely on a highly ordered and carefully regulated environment to maintain function and productivity. In the context of bioreactor design and scale-up, this emphasises the importance of preserving the hydrodynamic and multiphase flow conditions experienced by cells as processes transition from laboratory to industrial scale. Increases in reactor size inevitably introduce greater hydrodynamic complexity, spatial heterogeneities, and multiphase flow effects, which can alter circulation patterns, mixing behaviour, and local exposure histories. Preventing these scale-induced changes from adversely affecting cellular behaviour requires careful experimental and numerical strategies and an extensive characterisation of reactor hydrodynamics across scales.

Laboratory studies are inexpensive and controllable, but industrial performance is dictated by large-scale hydrodynamics that cannot be ignored. Scale-down approaches therefore play an essential role in bridging the gap between laboratory and production. By replicating the mixing conditions, hydrodynamic heterogeneities, and cell-exposure profiles of industrial-scale stirred tank reactors (STRs), they enable strain development, process optimisation, and risk reduction under manageable laboratory conditions, avoiding the cost and downtime of full-scale testing and thus saving considerable time and resources in the development of new biotechnological processes [Har17, Gau22, Pui23, Gau24, Aru25].

The use of scale-down approaches has steadily advanced over the past decades, with designs ranging from multi-compartment cascades to internally partitioned vessels, aimed at recreating the industrial scale conditions ranging from the mixing characteristics to the mass-transfer related exposures of the cells [Sch99, Neu10, Ana21]. Among these, the Single Multi-Compartment Bioreactor (SMCB) represents a promising concept, as it aims to mimic large-scale mixing characteristics through forced internal compartmentalisation. Current practice typically validates scale-down configurations by comparing global or local mixing times under single-phase flow, often using decolourisation methods [Gau22, Gau24]. While this provides a pragmatic basis, it neglects the fact that most industrial fermentations operate under aerated, multiphase conditions in which buoyancy-driven flows and bubble-induced mixing fundamentally alter circulation and compartmental exchange. As such, scale-down approaches based solely on single-phase metrics risk misrepresenting the cellular environment encountered at production scale.

A further dimension arises when considering how hydrodynamics are measured and characterised. Traditional Eulerian and single value metric approaches, such as global mixing time or volumetric mass transfer coefficients, are tied to spatially fixed frames of reference. These provide useful averages but may obscure the true heterogeneities that cells experience in situ [Ber24, Wei24]. In contrast, Lagrangian methods follow individual particles, or analogues of cells, along their lifelines through the reactor, capturing transient exposure histories to velocity fields, shear environments, and compartmental transitions. This cellular perspective is particularly powerful, as bioprocess performance ultimately depends on how cells perceive their environment, not on bulk averages [Kus17, Har23, Hof24].

Recent developments in numerical simulation, particularly the Lattice-Boltzmann Method coupled with Large-Eddy Simulation (LBM-LES), have made it possible to extend Lagrangian analysis to both laboratory and industrial scales. By combining experimental validation with numerical particle tracking, a much deeper understanding of mixing structures, circulation loops, and compartmentalisation under multiphase operation becomes possible [Hof25]. Lifeline analyses, supported by metrics such as Finite-Time Lyapunov Exponents (FTLE), offer a detailed and statistically robust quantification of cell-bioreactor interactions, moving beyond classical hydrodynamic metrics towards cell-centred analysis and scale-down approach.

The present thesis is embedded within the research grant *InterZell*, funded by the German Research Foundation (DFG) in its second funding period. The overarching programme investigates interactions at two fundamental levels. On the one hand, cell-cell interactions and on the other, cell-bioreactor interactions. This work is a product of the subproject *CHOLife+*, which is situated firmly in the latter category and specifically addresses the question of how bioreactor hydrodynamics shape cellular lifelines and productivity across scales. Within this framework, the thesis focuses on the development, application, and critical evaluation of Lagrangian, cell-centred characterisation methods for stirred tank reactors operating under both single- and multiphase conditions. While historically the scale-up and -down approaches have relied on global, ideally local mixing time studies, with local mixing times requiring determination numerically or experimentally with transparent acrylic reactors and not cell related metrics. This work establishes a consistent methodological framework for comparing hydrodynamic conditions across reactor scales from a Lagrangian perspective. By combining experimental techniques, such as Lagrangian Sensor Particles and decolourisation-based mixing studies, with validated numerical approaches using LBM-LES, the thesis develops and applies metrics that capture circulation behaviour, residence times, velocity distributions, and mixing intensities experienced by individual cells. This unified framework enables a scale-independent assessment of flow structures and cellular exposure histories, extending beyond traditional Eulerian and single-value metrics.

Within this methodological context, the Single Multi-Compartment Bioreactor (SMCB) is employed as a representative scale-down system to evaluate the applicability and limitations of current scale-down practices. The SMCB is not treated as a design to be developed or optimised, but as a test case for assessing how well existing scale-down configurations reproduce industrial-scale hydrodynamics when evaluated using multiphase, Lagrangian, cell-centred metrics. By applying identical experimental and numerical characterisation methods to an industrial 15 m^3 STR and its laboratory-scale counterpart, the thesis assesses the degree of hydrodynamic and cellular equivalence achievable with current scale-down approaches.

In doing so, this work demonstrates that scale-down strategies based solely on single-phase metrics, while useful, remain incomplete without multiphase and Lagrangian characterisation. The thesis emphasises the necessity of a cell-centred scaling approach based on lifelines, circulation and residence times, velocity distributions, and FTLE-based mixing metrics. By applying these methods consistently across scales, new insights are gained into how closely scale-down systems reflect the realities of large-scale operation. Ultimately, this advances the methodological basis for bioreactor scale transfer, enabling a more rigorous and biologically relevant, yet fluid-mechanically grounded, assessment of scaling concepts with respect to the cellular environment at production scale.

Chapter 2

Fundamentals and State of the Art

The following chapter addresses the fundamental theories and state of the art for the characterisation of stirred tank reactors on industrial scale. To start, the principles of stirred tank reactor design are laid out. Followed by, details on a variety of reactor characterisation methods of both Eulerian and Lagrangian nature. In addition to the experimental theory, an introduction into the numerical characterisation approach is made with details on different characterisation methods. Finally, reactor scale-down approaches for the recreation of large-scale mixing conditions on laboratory-scale are presented.

2.1 Fundamentals of Stirred Tank Reactor Design

Stirred tank reactors (STRs) are a cornerstone of the chemical and bioprocess engineering industry [Ari60]. Evolving from simple manually stirred containers to sophisticated systems designed for optimal mixing, heat, and mass transfer [Ari60, Nau07]. The widespread use of STRs in diverse industries, from pharmaceuticals to wastewater treatment, highlights their versatility and effectiveness in handling liquid- and multiphase processes.

The basic design elements of STRs are standardized to ensure consistency and interoperability across industries. Standards outlined in DIN 28130 form the fundamental basis for STR design and conceptualisation [Deu07]. From tank dimensions, types of agitators, baffles and auxiliary equipment. These standards provide guidelines for key aspects and requirements to ensure operational safety, efficiency and scalability. The essential components of an STR include:

- **Tank:** Typically a cylindrical vessel, often with a dished or flat bottom, designed to withstand high pressure and temperature variations.

- **Agitator:** A mechanical impeller system responsible for creating the desired mixing and dispersion of reactants within the tank.
- **Baffles:** Vertical flow breakers mounted on the interior walls of the tank to disrupt rotational flow and promote effective mixing.
- **Shaft:** Component which translates the energy input from the motor into the reactor allowing for smooth rotational motion.
- **Seals and Mountings:** Features that ensure a leak-proof system and maintain mechanical stability.
- **Ports and Nozzles:** Access points for reactants, sampling, instrumentation and utility connections.
- **Temperature control unit:** Auxiliary component responsible for the temperature control during operation.

These elements collectively ensure that STRs provide uniform mixing, control reaction kinetics, and facilitate heat and mass transfer. Not listed is the aeration unit generally required for bioprocesses to supply cultivation media with oxygen or other gases required for optimal operating conditions. The specifics to aeration units play a crucial role in biotechnology and form a close interplay with the respective impeller.

While the principles of STR design are universal, the specific configurations can be adapted to meet the requirements of various processes, such as single-phase mixing, gas-liquid reactions, solid suspension, or omitting baffles as is the case for many single-use bioreactors [Eib10, Eib11, Ber24]. Particularly the size of operation and application is a deciding factor for the configuration and design [Kra02, Deu07].

The standardisation allows for comparability between different applications and sizes of STR. To this end, in the following the most important fundamentals of the individual components are detailed based on one of the configurations used in this work. The basic configuration is shown through the schematic drawing in Figure 2.1.

The variable d_1 represents the diameter of the tank, the size varies based on the application, with laboratory-scale STRs as small as 5 cm [Epp25] to industrial STRs with diameters greater than 4.6 m [Zlo01]. The impeller diameter is denoted by d_2. The total tank height is h_{total} often being set in relation to tank diameter d_1 and often in a ratio between 1 and 5 [Zlo01]. The filling height is denoted as h_1, which varies depending on application and ratio of diameter to height of STR. Additionally, h_2 specifies the height of the first impeller from the STR floor and has a crucial role in the effective mixing and dispersion [Fit19].

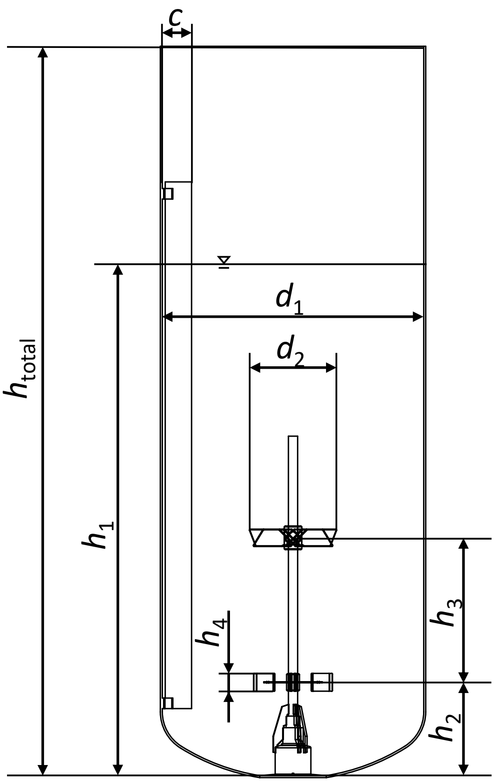


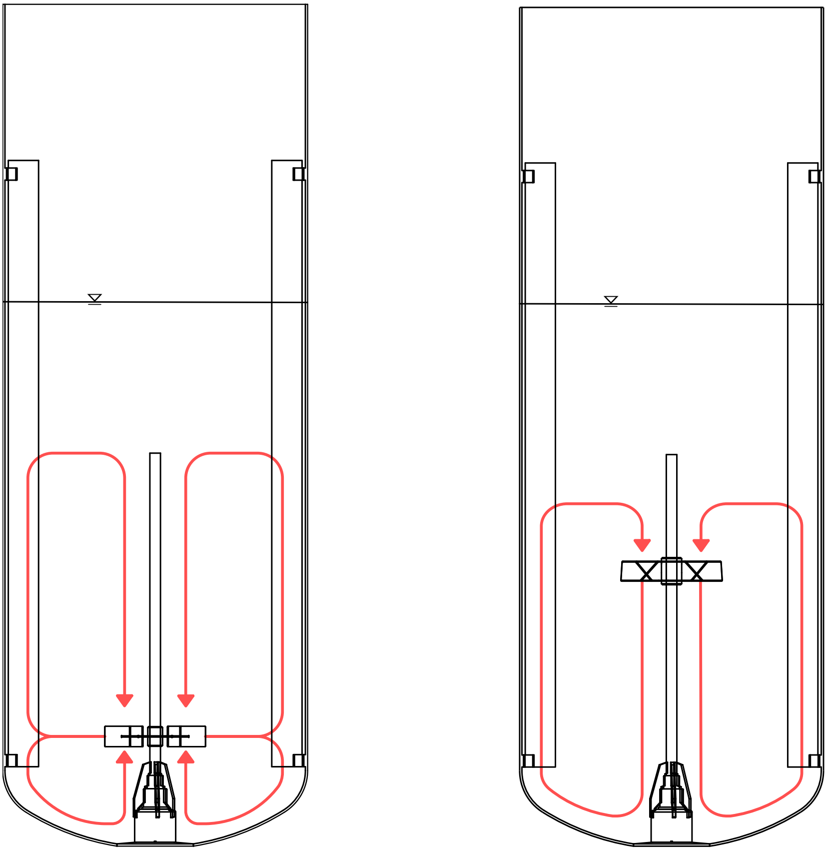
Figure 2.1 Schematic drawing of a stirred tank reactor with a two impeller configuration and details on the dimension nomenclature.

The variable h_3 refers to an intermediate distance between impellers 1 and 2 the distances strongly depend on the amount of impellers. Furthermore, h_4 indicates an impeller specific blade width. Finally, c denotes the baffle width [Kra02, Zlo01].

The effective mixing and dispersion of reactants and the media within the STR is crucial to its optimal operation. However, due to the strong variation in application there is no one-size fits all solution which is why certain aspects of the STR vary such as the reactor diameter or height. The size variations are however less defining than the influence the combination with different impeller configurations, baffles and aeration units have.

The influence of baffles is noticeable in the comparison between stainless steel and single-use STRs, as single use STRs often don't use baffles due to fabrication reasons. In return, there can be a strong vortexing of the fluid. Baffles enhance the mixing, prevent vortexing and promote intensification and turbulence within the STR. Variation in the aeration system can further affect the flow and mixing dynamics of a STR [Ros18]. The main influencing factor for flow pattern and mixing dynamics of a STR are connected to the impeller and the different combinations and variations [Eib10, Eib11, Ber24].

The main difference in impeller selection, can be broken-down to the impeller being axial or radial pumping. Common configurations for industrial applications often follow a combination of the two. The predicted flow patterns of the two variants are shown as red flow profiles in Figure 2.2.



(a) (b)

Figure 2.2 Schematic drawing of flow pattern for (a) radial pumping Rushton Turbine impeller and (b) axial pumping pitched blade.

The visualised flow patterns relate to the radial pumping impeller for which the Rushton Turbine is the most common, shown in Figure 2.2 (a). In this case the fluid is transported outwards radially to the STR wall where it separates into an upward and downward stream completes a loop and then return into the impeller region along the STR shaft. The axial pumping impeller on the other hand propels the fluid along one direction, much like the propeller of a plane. The circular motion of fluid seen in Figure 2.2 (b), is caused by the constraining STR walls and baffles allowing for a loop to be established, with a clear downward and upward flowing section without vortexing. The combination of the two can achieve unique and productive flow patterns in which the axial pumping impeller feeds the lower radial pumping impellers with streams flowing up along the baffles and generally down towards the STR shaft. The use of baffles is essential in such stirring processes, as if they are omitted, vortex formations develop, which can induce self-gassing, sloshing of the fluid, and ultimately a reduction in mixing performance.

To determine the most effective impeller, it is key to understand the present process requirements and have knowledge of physical properties and the media in the process. The Rushton turbine is the most commonly employed radial-pumping impeller for applications requiring high gas dispersion. Its design generates high shear rates, enabling effective breakup of the gas phase into many small bubbles. In contrast, axial-pumping impellers, such as the widely used pitched-blade or propeller impeller, are less efficient for gas dispersion. Instead, these impellers are preferred for processes in shear-sensitive environments propagating a good homogenisation over mechanical stress. In table 2.1 several common impellers are listed with various characteristics outlined, such as if they are situated close to the wall or more central off-wall or for what specific application they are most relevant and perform best and worst at indicated by the "+" and "-" respectively [Kre02, Hem03].

The main task of an impeller can be summed up to two actions, suspending and dispersing. Dispersion is a crucial factor in aerating a STR, as it involves the application of high shear stress fields to disperse different phases such as gas and liquid. Radial pumping impellers such as the Rushton Turbine are particularly well suited for such applications. While axial pumping impellers as listed in Table 2.1 thrive in areas of suspending, this is particularly important in the case of solid/liquid dispersion which is counteracted by the sedimentation of solids with a higher density than the media within the STR.

Temperature control is a key feature of STRs, especially in biotechnology and bioprocess engineering. Various methods are used to regulate temperature, such as a heated electric rod for lab-scale STRs, a large heated coil immersed in the reactor, a temperature-controlled double jacket or water bath connected to an external heat exchanger, or an external heated pumping loop [Zlo01, Kra02, Hem03].

Table 2.1 Summary of different characteristics for common impellers used in process engineering [Kre02, Hof24].

Name	**Propeller**	**Helical**	**Elefant Ear**	**Pitched Blade**	**Anker**	**Dissolver**	**Rushton Turbine**
Pumping mode	Axial				Radial		
Position	Off-wall	Near-wall	Off-wall	Off-wall	Near-wall	Off-wall	Off-wall
Homogenize	+++	+++	+++	+++	+++	—	—
Disperse Gas/Liquid	—	—	—	—	—	—	+++
Disperse Liquid/Liquid	—	—	—	—	—	+++	+++
Disperse Solid/Liquid	—	—	—	—	—	+++	—
Heat transfer	—	+++	—	+++	+++	—	—
Viscosity range (Pa s)	< 2	$< 10^3$	< 10			< 2	
Preferred Flow condition	Turbulent	Laminar	Transitional			Turbulent	