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Vorwort

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Hamburg, April 2026

*“Reißt die Ruder gegen das Meer
Es windet sich, es windet sehr
Die Welle schlägt gegen das Holz
Sie bricht, es bricht nich’ ihren Stolz
Den Hafen nie geseh’n, den Hafen nie vermisst
Wer kann es beweisen? Ich glaube, den Hafen gibt es nich’
Hab’ nie dem Nordlicht getraut, der Kompass zeigt nich’ nach Haus’
Brennende Leiber zu Tag, des Nachts ächzende Kälte und ich bleib’ gern, bleib’ hier
Ich bleibe junges Kind, damit ich weiß:
Wir sind unsinkbar, Kapitän!
Und bis meine Sonne sinkt, bleib’ ich Kind
Ich bleib’ gern, bleib’ hier

Sag kein Wort, Steuermann!
Was weißt du, wo ich enden kann?
Das Meer scheint leicht zu versteh’n
Das ist der Spiegel, auf dem wir Kreise dreh’n
Ich kann das Land nich’ seh’n, ich kann das Land nich’ hör’n
Die Matrosen um mich arbeiten fleißig an Streichhölzern
Hab mich in Stürmen gesonnt, hab ich mal fliegen gekonnt?
Hab wie der alte Pan längst vergessen, wo die ander’n Kinder sind
Ich bleib’ ewig junges Kind, damit ich weiß:
Wir sind unsinkbar, Kapitän!
Und bis meine Sonne sinkt, bleib’ ich Kind
Ich bleib’ gern, bleib’ hier

Zieh’ den Anker aus dem Sumpf
Er liegt schon ewig auf dem Grund
Hör’, das Volk ist längst verstummt
Zieh’ den Anker aus dem Sumpf.”*

— Mine, Anker (2016)

Abstract

Understanding complex hydrodynamics in bioreactors is essential for optimizing bioprocesses, particularly with respect to spatial and temporal heterogeneities in nutrient supply. Conventional fixed sensor probes provide time-resolved but spatially limited measurements of key fermentation parameters such as temperature, pH, or dissolved oxygen concentration, leaving spatial gradients undetected. As a result, it remains unclear how long cells are exposed to specific environments and how this affects product quality and yield. To overcome this issue, a detailed fluid dynamic characterization of the bioreactor is required, including the derivation of cell lifelines, which are spatiotemporal profiles of the local environment that individual cells encounter during their journey through a reactor. Such lifelines aim to predict the performance and metabolism of production cell lines such as immunoglobulin IgG1-producing Chinese Hamster Ovary (CHO) cells. To enable lifeline measurements on industrial scale, Lagrangian Sensor Particles (LSP) offer a solution. These encapsulated, instrumented particles act as mobile *in situ* probes and can carry multiple sensors to measure temperature, pH, or chemical composition. However, due to their relatively large size and inertia, LSPs do not follow the flow as cells do because they exceed the smallest scales of the flow. This raises the question of how accurately they represent cell lifelines and which concentration heterogeneities they can capture. This thesis presents three complementary studies to assess the capabilities and limitations of LSPs. These include numerical simulations, laboratory-scale Lagrangian particle tracking experiments, and industrial-scale LSP measurements. Together, these approaches investigate the flow-following behavior of LSPs, their ability to resolve hydrodynamic compartments in bioreactors, and their potential to provide industrial-scale fluid dynamic data for the development of scale-down models.

In the first part of this thesis, highly spatiotemporally resolved four-dimensional flow measurements utilizing 4D-Particle Tracking Velocimetry (4D-PTV) in a 3 L stirred tank reactor (STR) investigate two inertial particle types of different sizes, made from polyethylene and calcium alginate, under various impeller frequencies. The comparatively larger alginate beads replicate LSPs in a larger STR. Complementary Lattice-Boltzmann large eddy simulations (LB LES), which model the respective particle trajectories, are compared with and validated by the experimental data. Time-averaged Eulerian data agree well between

Abstract

experiments and simulations, while Lagrangian results show discrepancies for the alginate beads at high velocities and accelerations. Micro-scale Stokes numbers are found to significantly exceed the flow-following criterion of $St \leq 0.01$, particularly for the larger particles representing LSPs. The analysis further identifies a theoretical maximum particle diameter of approximately 1 mm as the upper limit that still meets the flow-following criterion for LSPs on industrial scale.

Scaling up to industrial bioreactors, the second part of this thesis implements developed LSPs with a diameter of $d_{LSP} = 40$ mm equipped with a pressure sensor within a 15,000 L STR. With an estimated Stokes number of $St \approx 0.004$ on the macro-scale, utilized LSPs indicate flow-following behavior for large length scales. Analyses of axial probability distributions, axial velocities, circulation times, and residence times reveal the ability of LSPs to detect three hydrodynamic compartments and potential oxygen limitation zones. For the first time, the three-staged Elephant Ear stirrer configuration is characterized by dimensionless flow numbers, which agree with literature values and mixing time scaling laws.

In the third part of this thesis, experimental LSP trajectories are compared with LB LES of replicated LSPs and massless tracers in the same 15,000 L STR, equipped with a Rushton turbine and a pitched blade turbine. The results show differences in the axial probability of presence and velocities, which can be attributed to factors such as the chosen grid resolution and the higher number of particles in the simulations, leading to particle collisions or perturbations. However, both experimental and simulated data confirm the compartmentalized flow structure, which is further validated by local mixing time measurements. Results again indicate a theoretical upper particle size limit necessary to be flow-following on the micro-scale, similar to the outcomes of the first study. Experimental LSPs effectively capture macro-scale and meso-scale hydrodynamics with Stokes numbers of $St \approx 0.002$ and $St \approx 0.2$, respectively. This emphasizes the capability of the current LSP technology to study hydrodynamics at scales comparable to or larger than the impeller in an industrial bioreactor.

The joint utilization of an advanced experimental particle tracking technique such as 4D-PTV, obtained trajectory results from state-of-the-art LSPs, complemented by computationally demanding LB LES, offers a comprehensive toolbox for obtaining Lagrangian particle trajectories, hydrodynamic compartments, and mixing behavior across scales. Insights into compartmentalization and flow regimes gained through these approaches not only highlight the potential of LSPs as an experimental technique for providing industrial-scale fluid dynamic data to support scale-down model development but also reveal opportunities to optimize both LSP design and bioreactor setup. This facilitates improved nutrient and oxygen distribution, ultimately enhancing cell viability and process efficacy.

Zusammenfassung

Eine zentrale Voraussetzung für die Optimierung biotechnologischer Prozesse ist das Verständnis über die komplexe Hydrodynamik in Bioreaktoren. Besonders wichtig sind dabei räumliche und zeitliche Heterogenitäten in der Nährstoffversorgung, die mit klassischen, ortsfesten Sensoren kaum erfassbar sind. Zwar liefern diese kontinuierliche Messwerte zu Parametern wie Temperatur, pH oder Sauerstoffkonzentration, sie berücksichtigen jedoch keine räumlichen Gradienten. Dadurch bleibt unklar, wie lange Zellen bestimmten Umgebungsbedingungen ausgesetzt sind und welchen Einfluss dies auf die Produktqualität und -ausbeute hat. Eine fluiddynamische Charakterisierung des Reaktors ermöglicht die Ableitung sogenannter Zelltrajektorien, im Folgenden als *lifelines* bezeichnet. Diese *lifelines* liefern raum-zeitliche Profile der lokalen Umgebungsbedingungen, die einzelne Zellen im Reaktor erfahren, und erlauben Rückschlüsse auf Stoffwechsel und Leistungsfähigkeit industriell genutzter Zelllinien wie IgG1-produzierender CHO-Zellen (englisch: Chinese Hamster Ovary). Ein Ansatz zur Erfassung solcher Trajektorien im industriellen Maßstab sind Lagrangesche Sensor Partikel (LSP). Diese verkapselten Partikel lassen sich mit verschiedenen Sensoren ausstatten und sind somit *in situ* Messsonden, die sich frei im Reaktor bewegen können. Aufgrund ihrer relativ großen Größe und der daraus resultierenden Trägheit folgen LSPs dem Strömungsfeld nicht exakt wie Zellen. Daraus ergibt sich die Frage, wie genau sie *lifelines* abbilden und welche Strukturen und Heterogenitäten sie erfassen können. Diese Arbeit untersucht in drei komplementären Studien die Möglichkeiten und Grenzen von LSPs. Neben numerischen Simulationen werden Lagrangesche Partikel-Tracking-Experimente im Labormaßstab sowie Messungen mit LSPs in einem industriellen Reaktor durchgeführt. Diese Ansätze ermöglichen die Untersuchung des Strömungsverhaltens von LSPs, ihrer Fähigkeit zur Erkennung hydrodynamischer Kompartimente in Bioreaktoren und ihres Potenzials, strömungsmechanische Daten im industriellen Maßstab für die Entwicklung von Scale-Down-Modellen bereitzustellen.

Im ersten Teil dieser Arbeit werden hochaufgelöste vierdimensionale Strömungsmessungen mittels 4D-Particle Tracking Velocimetry (4D-PTV) in einem 3 L Rührkesselreaktor (STR) durchgeführt. Dabei werden zwei inertielle Partikeltypen unterschiedlicher Größe, bestehend aus Polyethylen und Calciumalginat, unter verschiedenen Rührerdrehzahlen unter-

sucht. Die vergleichsweise größeren Alginatpartikel dienen als Modell für LSPs in einem größeren STR. Ergänzend werden Lattice-Boltzmann Large Eddy Simulationen (LB LES) durchgeführt, die die Partikeltrajektorien abbilden und mit den experimentellen Daten verglichen sowie validiert werden. Während die zeitlich gemittelten eulerschen Daten in Experimenten und Simulationen gut übereinstimmen, zeigen sich bei den Alginatpartikeln in der Lagrangeschen Auswertung deutliche Abweichungen bezüglich hoher Geschwindigkeiten und Beschleunigungen. Die ermittelten mikroskaligen Stokes-Zahlen überschreiten das Strömungsfolge-Kriterium $St \leq 0,01$ deutlich, insbesondere für die Alginatpartikel. Für den industriellen Maßstab ergibt sich daraus eine theoretische maximale LSP Größe von etwa 1 mm, die dieses Strömungsfolge-Kriterium noch erfüllen würde.

Im industriellen 15.000 L-Maßstab werden im zweiten Teil dieser Arbeit entwickelte LSPs mit einem Durchmesser von $d_{LSP} = 40\text{ mm}$ und integriertem Drucksensor eingesetzt. Mit einer geschätzten Stokes-Zahl von $St \approx 0,004$ auf der Makroskala zeigen die LSPs ein ausreichendes Strömungsfolgeverhalten für große Längenskalen. Analysen der axialen Aufenthaltswahrscheinlichkeiten, axialen Geschwindigkeiten, Zirkulations- und Verweilzeiten belegen, dass LSPs in der Lage sind, drei hydrodynamische Kompartimente sowie potenzielle Zonen mit einer Sauerstofflimitierung zu identifizieren. Erstmals wird zudem die dreistufige Elephant-Ear-Rührer-Konfiguration über dimensionslose Pumpkennzahlen charakterisiert, die gut mit Literaturwerten und Mischzeitskalierungen übereinstimmen.

Im dritten Teil der Arbeit werden LSP Experimente mit LB LES verglichen, in denen sowohl LSPs als auch masselose Tracer abgebildet werden. Hierzu wird abermals der 15.000 L STR genutzt, nun mit einer Konfiguration aus Scheiben- und Schrägblatt-Rührer. Die Ergebnisse zeigen Unterschiede in der axialen Aufenthaltswahrscheinlichkeit und den Geschwindigkeiten, die auf Faktoren wie die gewählte Gitterauflösung und die höhere Partikelanzahl in den Simulationen zurückzuführen sind. Dennoch bestätigen sowohl experimentelle als auch simulierte Daten die Kompartimentierung der Strömung, die zusätzlich durch lokale Mischzeitmessungen validiert wird. Ähnlich zur ersten Studie, weisen die Ergebnisse erneut auf eine theoretische Obergrenze der LSP-Größe hin, die erforderlich ist, um mikroskaligen Strömungen zu folgen. Experimentelle LSPs erfassen zuverlässig die Hydrodynamik auf Makro- und Mesoskala mit Stokes-Zahlen von $St \approx 0,002$ bzw. $St \approx 0,2$ und verdeutlichen die Eignung der aktuellen LSP-Technologie, Strömungen in industriellen Bioreaktoren auf Skalen zu untersuchen, die gleich oder größer als der Rührerdurchmesser sind.

Die kombinierte Nutzung einer hochaufgelösten experimentellen Partikel-Tracking-Methode wie 4D-PTV, der Trajektorienmessungen mit LSPs und ergänzender, rechenintensiver Simulationen bietet ein umfassendes Methodenportfolio zur Bestimmung Lagrangescher Partikeltrajektorien, hydrodynamischer Kompartimente und skalenübergrei-

fenden Mischverhaltens. Die gewonnenen Erkenntnisse zur Kompartimentierung und zu Strömungsregimen zeigen nicht nur das Potenzial von LSPs als experimentelle Methode zur Bereitstellung großskaliger Strömungsdaten für die Entwicklung von Scale-down-Modellen auf, sondern eröffnen auch Möglichkeiten zur Optimierung des LSP-Designs sowie der Reaktorkonfiguration. Dies unterstützt eine verbesserte Verteilung von Nährstoffen und Gelöstsauerstoff und trägt letztlich zur Steigerung der Zellviabilität und Prozesseffizienz bei.

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Nomenclature

Arabic symbols

$\mathbf{c}_i$	$\text{m} \cdot \text{s}^{-1}$	Discrete lattice velocity vector
$\mathbf{d}_i$	–	Disparity vector for camera i
$\mathbf{F}$	$\text{kg} \cdot \text{m} \cdot \text{s}^{-2}$	Force vector
$\mathbf{F}_D$	$\text{kg} \cdot \text{m} \cdot \text{s}^{-2}$	Drag force vector
$\mathbf{F}_{\text{ext}}$	$\text{kg} \cdot \text{m} \cdot \text{s}^{-2}$	External force vector
$\mathbf{F}_L$	$\text{kg} \cdot \text{m} \cdot \text{s}^{-2}$	Lift force vector
$\mathbf{F}_p$	$\text{kg} \cdot \text{m} \cdot \text{s}^{-2}$	Particle-particle interaction force vector
$\mathbf{u}$	$\text{m} \cdot \text{s}^{-1}$	Fluid velocity vector
$\mathbf{u}'_{\text{rms}}$	$\text{m} \cdot \text{s}^{-1}$	Root mean square of fluid velocity fluctuation
$\mathbf{V}$	$\text{m} \cdot \text{s}^{-1}$	Slip velocity vector
$\mathbf{v}$	$\text{m} \cdot \text{s}^{-1}$	Particle velocity vector
$\mathbf{x}$	m	Spatial position vector
$\bar{I}'_t$	–	Normalized average gray-scale intensity over time
$\bar{I}_0$	–	Average gray-scale intensity at experiment start
$\bar{I}_\infty$	–	Average gray-scale intensity at full homogenization
$\bar{I}_t$	–	Average gray-scale intensity over time
$\bar{S}$	s^{-1}	Resolved rate-of-strain tensor

Nomenclature

$\tilde{I}'_t$	–	Normalized average gray-scale intensity over time in specific pixel array
$\tilde{I}_{0,l,m}$	–	Average gray-scale intensity at experiment start in pixel array (l, m)
$\tilde{I}_{\infty,l,m}$	–	Average gray-scale intensity at full homogenization in pixel array (l, m)
$\tilde{I}_{t,l,m}$	–	Average gray-scale intensity over time in pixel array (l, m)
a	–	Parameter for general drag correlation
A_c	–	Acceleration parameter
A_{ex}	m^2	Exchange area
Ar	–	Archimedes number
b	–	Parameter for general drag correlation
C	$S \cdot m^{-1}$	Conductivity
c	–	Parameter for general drag correlation
c_D	–	Drag coefficient
c_{gas}	$mol \cdot L^{-1}$	Dissolved gas concentration
C_S	–	Smagorinsky constant
d	–	Parameter for general drag correlation
d_{imp}	m	Impeller diameter
d_{LSP}	m	LSP diameter
$d_{p,th}$	m	Theoretical maximum particle diameter
d_p	m	Particle diameter
f	$m^{-6} \cdot s^3$	Particle distribution function
f^{eq}	$m^{-6} \cdot s^3$	Equilibrium particle distribution function
g	$m \cdot s^{-2}$	Gravitational acceleration

h	m	Reactor height
i	–	Horizontal pixel or number of cameras
j	–	Vertical pixel
k	m^{-1}	Wave number
L	m	Characteristic length scale
l, m	–	Exemplary pixel position in array
L_E	m	Taylor length scale
LX	–	Number of lattice points
m	kg	Mass
M'_i	–	New mapping function of camera i
m_f	kg	Mass of fluid
m_p	kg	Mass of particle
M_i	–	Initial mapping function of camera i
n	s^{-1}	Impeller frequency
N_A	mol^{-1}	Avogadro constant
P	$\text{kg} \cdot \text{m}^2 \cdot \text{s}^{-3}$	Global power input
p	$\text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-2}$	Pressure
PV^{-1}	$\text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-3}$	Global power per volume input
$q_{s,\max}$	$\text{mol} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$	Maximum specific substrate uptake rate
q_s	$\text{mol} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$	Specific substrate uptake rate
Re_{STR}	–	Stirrer Reynolds number
Re_p	–	Particle Reynolds number
St	–	Stokes number
T	$^{\circ}\text{C}$	Temperature

Nomenclature

t	s	Time
u	$\text{m} \cdot \text{s}^{-1}$	Velocity of the fluid
V	m^3	Reactor working volume
v	$\text{m} \cdot \text{s}^{-1}$	Particle velocity
v_{mag}	$\text{m} \cdot \text{s}^{-1}$	Particle velocity magnitude
X	—	Acid/base ratio
x'_i	—	Shifted horizontal pixel coordinate on sensor of camera i
x_i	—	Exemplary horizontal pixel coordinate on sensor of camera i
y'_i	—	Shifted vertical pixel coordinate on sensor of camera i
y_i	—	Exemplary vertical pixel coordinate on sensor of camera i

Greek symbols

ζ	$\text{m} \cdot \text{s}^{-1}$	Particle velocity vector
$\Delta\rho$	$\text{kg} \cdot \text{m}^{-3}$	Density difference
Δx	m	Lattice spacing or filter width
η	$\text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-1}$	Dynamic viscosity
Λ	m	Integral length scale
λ_f	m	Kolmogorov length scale
λ_k	m	Kolmogorov length scale
μ	s^{-1}	Specific cell growth rate
ν	$\text{m}^2 \cdot \text{s}^{-1}$	Kinematic viscosity
ν_t	$\text{m}^2 \cdot \text{s}^{-1}$	Sub-grid eddy viscosity
Ω	$\text{m}^{-6} \cdot \text{s}^2$	Collision parameter
$\bar{\varepsilon}_T$	$\text{m}^2 \cdot \text{s}^{-3}$	Mean energy dissipation rate

ϕ	–	Sphericity
ρ_f	$\text{kg} \cdot \text{m}^{-3}$	Density of fluid
ρ_p	$\text{kg} \cdot \text{m}^{-3}$	Density of particle
σ^2	–	Variance
τ	s	Relaxation time, integration variable, or residence time
τ_C	s	Residence time in center compartment
τ_L	s	Residence time in lower compartment
τ_p	s	Particle relaxation time
τ_U	s	Residence time in upper compartment
τ_f	s	Time characteristic of the flow field
$\Theta_{\text{glob},95,U}$	s	Global mixing time in upper compartment after 95 % mixedness
$\Theta_{\text{glob},95}$	s	Global mixing time after 95 % mixedness
$\Theta_{\text{glob},99}$	s	Global mixing time after 99 % mixedness
$\Theta_{\text{loc},95}$	s	Local mixing time after 95 % mixedness

Dimensionless numbers

$Ar = \frac{\rho_f (\rho_p - \rho_f) g d_p^3}{\eta^2}$	Archimedes number
$Re_p = \frac{\rho_f d_p V}{\eta}$	Particle Reynolds number
$Re_{\text{STR}} = \frac{\rho_f n d_{\text{imp}}^2}{\eta}$	Stirrer Reynolds number
$St = \frac{\tau_p}{\tau_f}$	Stokes number

Abbreviations

2D	2-dimensional
3D	3-dimensional
4D-PTV	4D-Particle Tracking Velocimetry

Nomenclature

BGK	Bathnagar, Gross, Krook model equation
BTB	Bromothymol blue
CFD	Computational fluid dynamics
CHO	Chinese hamster ovary
CO ₂	Carbon dioxide
CRISPR	Clustered regularly interspaced short palindromic repeats
CTD	Circulation time distribution
D3Q19	Velocity vector model structure
DFG	Deutsche Forschungsgemeinschaft
DNS	Direct numerical simulation
DO	Dissolved oxygen
FDA	U.S. Food & Drug Administration
GPS	Global-Positioning System
GPU	Graphics processing unit
H ₂	Hydrogen
H ₂ S	Hydrogen sulfide
HCl	Hydrochloric acid
IBM	Immersed boundary method
IBVT	Institute of Biochemical Engineering
IMU	Inertial Measurement Unit
IoT	Internet of Things
IPR	Iterative particle reconstruction
LB LES	Lattice-Boltzmann large eddy simulation

LBGK	Lattice Bathnagar, Gross, Krook model equation
LDA	Laser Doppler Anemometry
LED	Light-emitting diode
LES	Large eddy simulation
LiDaR	Light detection and ranging
LPT	Lagrangian particle tracking
LSP	Lagrangian Sensor Particle
MD	Molecular dynamics
MMR	Magneto-mechanical resonator
NaOH	Sodium hydroxide
NTC	Negative-temperature-coefficient
O ₂	Oxygen
OTF	Optical transfer function
PBT-6	6-bladed pitched blade turbine
PE	Polyethylene
PEEK	Polyether ether ketone
PIV	Particle Image Velocimetry
ppp	Particles per pixel
PTV	Particle Tracking Velocimetry
RaDaR	Radio detection and ranging
RANS	Reynolds-averaged Navier-Stokes
RGB	Red, green, blue color model
RQ	Research question
RT-6	6-bladed Rushton turbine

Nomenclature

RTD	Residence time distribution
SGS	Sub-grid scale
SMCB	Single multicompartment bioreactor
SPP	Priority program (Schwerpunktprogramm)
STB	Shake-The-Box
STR	Stirred tank reactor
Tomo-PIV	Tomographic Particle Image Velocimetry
VCC	Viable cell count
VSC	Volumetric self-calibration

Chapter 1

Introduction

"The revolutions in biotech and infotech will give us control of the world inside us, and will enable us to engineer and manufacture life. [...] Nobody knows what the consequences will be. Humans were always far better at inventing tools than using them wisely."
from Yuval Noah Harari [Har19].

The historian Harari suggests that the developments and convergence in biotechnology (biotech) and information technology (infotech) will fundamentally reshape human society, economies, and even the definition of what it means to be human. Advances in data processing and artificial intelligence allow us to decode and manipulate biological systems with unprecedented precision. Curing diseases by genetic engineering with breakthroughs like CRISPR (clustered regularly interspaced short palindromic repeats) transforms an organism into a data processing system. By decoding the algorithms governing biological processes, biotechnology influences life forms, which blurs the line between natural and artificial life. A striking example is the development of AlphaFold, a neural network that achieved predictions of three-dimensional protein structures with atomic accuracy. This machine learning approach, which integrates physical and biological knowledge, surpassed decades of experimental work by predicting the structures of millions of proteins within years, even if no such structure is known [Jum21]. Eventually, this can lead to an improved quality of life, including eradicating diseases, food security, and sustainability. However, this comes with a high ethical responsibility, as developments could worsen social and economic inequality if they remain accessible only to the wealthy. It is of essence that ethical frameworks guide scientific progress and that governments ought to continue prioritizing public welfare by funding foundational scientific research. In that case, public-private partnerships have a high potential to tackle global challenges such as climate change, famine, or pandemics, but they

Introduction

need to be monitored by governments and ensure that biotechnological advances benefit humanity as a whole.

The German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) is Germany's leading organization for funding independent, highest-ranking, and foundational scientific research with a current annual budget of over 3.8 billion Euro in 2024 [Deu24]. Their Code of Conduct "Safeguarding Good Research Practice" represents the fundamental principles and standards of good research practice [Deu22]. By supporting projects initiated by the academic community, the DFG fosters interdisciplinary innovation and excellence, including biotechnology programs exploring scalable and sustainable production processes. In the context of climate change and resource shortages, biotechnology can contribute a great deal to the transition to a circular economy. Biotechnological processes, which often rely on renewable resources, can enhance sustainable production methods and help mitigate environmental impacts. Synthetic biology provides new possibilities for the modification and enhancement of existing cell lines through genetic engineering of strains and even merging different production hosts in one reaction volume, such as bacteria, yeast, and fungi, along with mammalian cells such as Chinese hamster ovary (CHO) cells. To that end, interestingly, 99 % of microbial flora, referred to as "biological dark matter", remain unexplored and uncultured, yet show promising potential to increase yields for efficient bio-based products [Sch23b, Ber18].

Objectives and research methods

In terms of this topic, in 2019, the DFG approved funding for the priority program "Novel Production Processes through Cross-Scale Analysis, Modeling and Design of Cell-Cell and Cell-Bioreactor Interactions" (InterZell SPP 2170), which is coordinated by Prof. Dr.-Ing. Ralf Takors (Institute of Biochemical Engineering, University of Stuttgart) and comprises eleven subprojects with a total of 22 research associates [Ins25]. The program is designed to create a new research topic and facilitate collaboration among researchers. Its primary objective is to advance fundamental knowledge of how microbial cell-to-cell and cell-to-bioreactor interactions contribute to production processes. By understanding these interactions and developing methods to measure them, the program seeks to enable the creation of new and more efficient production pathways for the future. To do so, the focus of the SPP 2170 program is to profit from synthetic co-cultures of microorganisms, including genetically modified strains, to optimize the production of valuable target products under controlled bioreactor conditions, scaling up from laboratory conditions to industrial applications, addressing challenges in multi-scale bioprocesses and understanding occurring interactions. Hence, the program

provides a unique opportunity to collaborate across disciplines (metabolic engineering, bio-engineering, microbial engineering, bioinformatics, modeling, fluid mechanics, etc.) and bridges the gap between small-scale laboratory work and industrial processes by developing practical solutions. If these solutions can be successfully scaled from laboratory to industrial scale, they will have a significant impact, and thus, they can lead to the creation of products that benefit society. This topic is of such significance that an entire special issue in the journal "Engineering in Life Sciences" has been dedicated to it [Tak22].

However, as with any research, there are numerous obstacles to overcome. Fundamental research, especially in areas that are not yet fully understood, often presents unknown challenges. In the SPP 2170, obstacles include issues related to the identification of microbial cell interactions while cultivating, the selection of appropriate microbial groups for specific conditions, and the replication of large-scale production environments in a controlled laboratory setting. The major complex difficulty lies in translating the conditions observed on a laboratory scale to the much more stressful conditions on industrial scale, and vice versa.

To address this issue, the subproject "CHOLife" in the SPP 2170 investigates the scale-up and scale-down challenges by combining the utilization of a 15,000 L acrylic glass bioreactor (Institute of Multiphase Flow, Hamburg University of Technology) with the development of a novel scale-up device (Institute of Biochemical Engineering, IBVT, University of Stuttgart), ultimately aiming to predict the industrial performance and metabolism of immunoglobulin IgG1-producing CHO cells. A key aspect is the experimental and numerical multi-scale analysis, which examines critical factors for cell growth. This includes investigating spatial and temporal gradients in the bioreactor, as well as the cell's time-resolved trajectory through these heterogeneities. Changes in dissolved gas concentration, pH, or other substrates can influence product quality and yield, and play a key role in bioprocess performance. In particular, oxygen is a crucial component for cell growth and must be efficiently supplied in large-scale reactors. However, the oxygen concentration, originating from bubbles and the characteristic mass transfer across their micro-scale boundary layers into the cultivation volume, varies along the trajectory of a single cell throughout the reactor. This leads to certain stress conditions in the cell and potential changes in its metabolism and expression.

Within the subproject "CHOLife", this thesis elaborates and discusses various approaches of how flow trajectories are measured in laboratory- and industrial-scale bioreactors to detect hydrodynamic heterogeneities and eventually transfer and integrate those multi-scale flow structures into a novel scale-down reaction system at the IBVT.

Chapter 2

Theoretical background

This chapter provides the theoretical background relevant to understanding mixing heterogeneities in industrial-scale bioreactors, and how a multi-scale fluid dynamic analysis using lifelines can be applied to investigate and mitigate performance losses during scale-up or biotechnological process development. It introduces key experimental methods such as mixing time measurements and particle tracking, followed by advancements in mobile sensor technology with a focus on Lagrangian Sensor Particles (LSP) and their application in biotechnology, particularly on industrial scale. As experiments are accompanied by numerical simulations, the foundations and governing equations of Lattice-Boltzmann large eddy simulations for particle-laden flows are outlined, along with an overview of particle types simulated in this work. Additionally, the distinction between Eulerian and Lagrangian evaluation frameworks, relevant dimensionless numbers, and characteristic length scales are presented to support a multi-scale analysis approach. Finally, the concept of particle lifelines is introduced as a tool to transform the knowledge about hydrodynamic compartments by translating large-scale heterogeneities into meaningful insights for scale-down experiments. Each topic builds toward evaluation strategies, outcomes and interpretations presented in the subsequent chapters.

2.1 Mixing heterogeneities in bioreactors

Large-scale biopharmaceutical production processes, such as those involving Chinese hamster ovary (CHO) cells in stirred tank reactors (STR) with working volumes of up to 20,000 L or more, are highly prone to the formation of spatial gradients, which correspond to inhomogeneous distributions of compounds or parameters within the reactor (e.g. pH, temperature, shear rate) [Nad21, Lar06], as exemplary seen in Figure 2.1. On the left, Figure 2.1 a) shows an exemplary colorimetric mixing time trial with bromothymol blue (BTB) in a 15,000 L STR

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made of acrylic glass after the addition of an acidic solution, where distinct compartments can be observed. On the right, Figure 2.1 b) presents an instantaneous fluid velocity heat map from a numerical simulation of the same reactor utilizing M-Star CFD. In the vicinity of the impellers, and depending on the respective flow direction, high velocities occur, while flow velocity decreases with increasing distance. In the upper region of the reactor, fluid velocities are the lowest, leading to delayed homogenization and mass transfer compared to regions near the impellers.

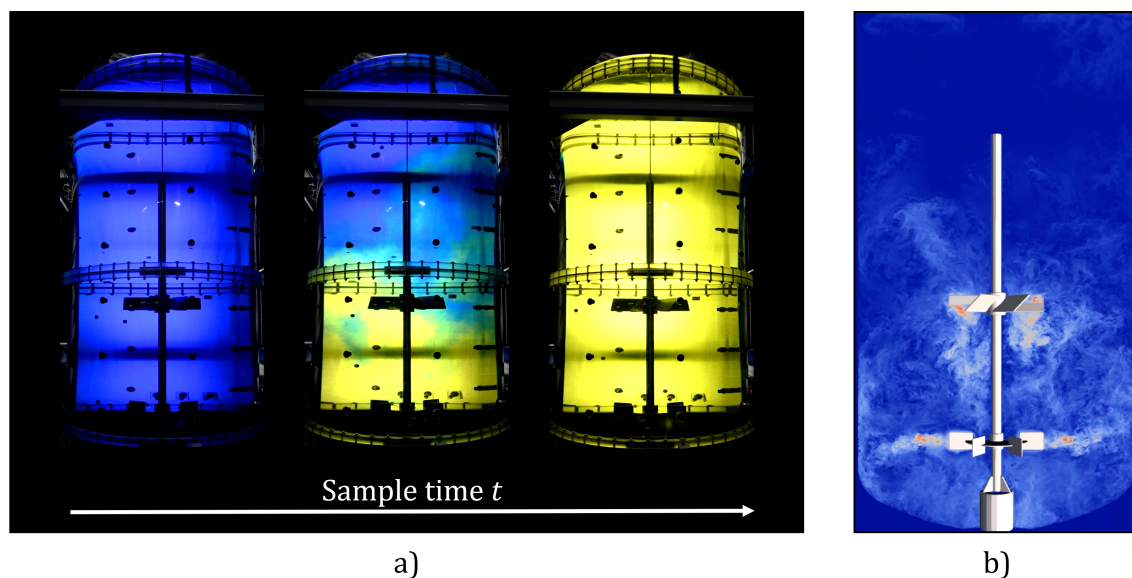


Figure 2.1: a) Exemplary depiction of a mixing time trial with bromothymol blue in an acrylic glass bioreactor with $V = 12,500\text{L}$, after 0 s , 65 s , and 160 s of the addition of hydrochloric acid; b) Exemplary depiction of an accompanying Lattice-Boltzmann large eddy simulation by means of M-Star CFD of the identical reactor demonstrating velocity gradients, such as high and intermediate fluid velocities in the lower half of the reactor and low fluid velocities in the upper half of the reactor.

Generally, in case of cell cultivations, substrate gradients tend to develop once the uptake rate exceeds the transport rate [Swe87]. On laboratory scale and during the development of novel bioprocesses, these heterogeneities are either comparatively unlikely to occur or appear in a different spatial and temporal manner, since large-scale chaotic motion often differs significantly in both scenarios. However, during process scale-up, such gradients will lead to a decline of critical key performance criteria, such as a decrease in product quantity and quality [Bru17]. Mixing times in STRs, as demonstrated in Figure 2.1 a), provide the duration required for the utilized setup to homogenize different phases (e.g. gas/liquid) or multiple single-phase contents (e.g. liquid/liquid) to a desired degree of homogeneity.

2.1 Mixing heterogeneities in bioreactors

In industrial mixing and in terms of the characterization of STRs, the 95 % mixing time criterion is a commonly used degree in order to classify an STR as adequately mixed [Pau04, Kra12]. One possible method to determine the mixing time in a bioreactor is the colorimetric approach by means of a pH-sensitive dye and the addition of a chemical that removes or changes the color (e.g. by the addition of acid and base). Hence, insufficiently mixed areas can be identified as they retain the initial color, in contrast to the remaining STR, which has undergone a color change [Pau04, Ros18, Cab07, Fit21]. To perform such an analysis, it is assumed that the reactor is symmetrical, and a two-dimensional (2D) projection sufficiently represents the volume for the evaluation [Fit21].

A recent study by Fitschen et al. [Fit21] presented and discussed the sensitivity of the acid/base ratio X during a colorimetric mixing time experiment emphasizing the neutralization reaction on microscopic scale. On the molecular level, in the case of $X = 1 : 1$, the reactor can be considered fully homogenized only when each acid molecule is able to encounter and neutralize a corresponding base molecule. This condition is particularly vital for the comprehensive understanding and characterization of mixing equipment in processes involving fast chemical reactions, where the degree of molecular mixing governs the overall reaction rate [Bal99]. However, macroscopic mixing times are more relevant for long-term biotechnological processes such as a CHO cell cultivation. In this context, a macroscopic characterization of the STR is sufficient for the purposes of this study. Accordingly, an acid/base ratio of $X = 2 : 1$ is utilized in this work, which enables comparison with literature data and facilitates assessment of overall substrate availability by revealing poorly mixed regimes and compartments, based on a 2D projection.

Colorimetric global mixing time measurements

In this work, the global mixing time is determined by the colorimetric approach utilizing bromothymol blue (BTB) as a pH indicator. BTB changes color from blue to yellow upon protonation by means of a 2 M hydrochloric acid (HCl) solution (Fig. 2.1 a)). The color shift can be reversed by adding a 2 M sodium hydroxide (NaOH) solution. The experimental setup and procedure is described in detail in Chapter 6.

In order to evaluate the global mixing, the average gray-scale intensity progression $\bar{I}_t$ is calculated from the RGB-camera images as the mean over all pixels of interest. Hence, the global mixing time represents an overall average for the entire system or for a defined volume of interest. To do so, as a reference, the respective average gray-scale intensity at the start of the experiment $\bar{I}_0$ and at the point of full homogenization $\bar{I}_\infty$ is taken in order to calculate

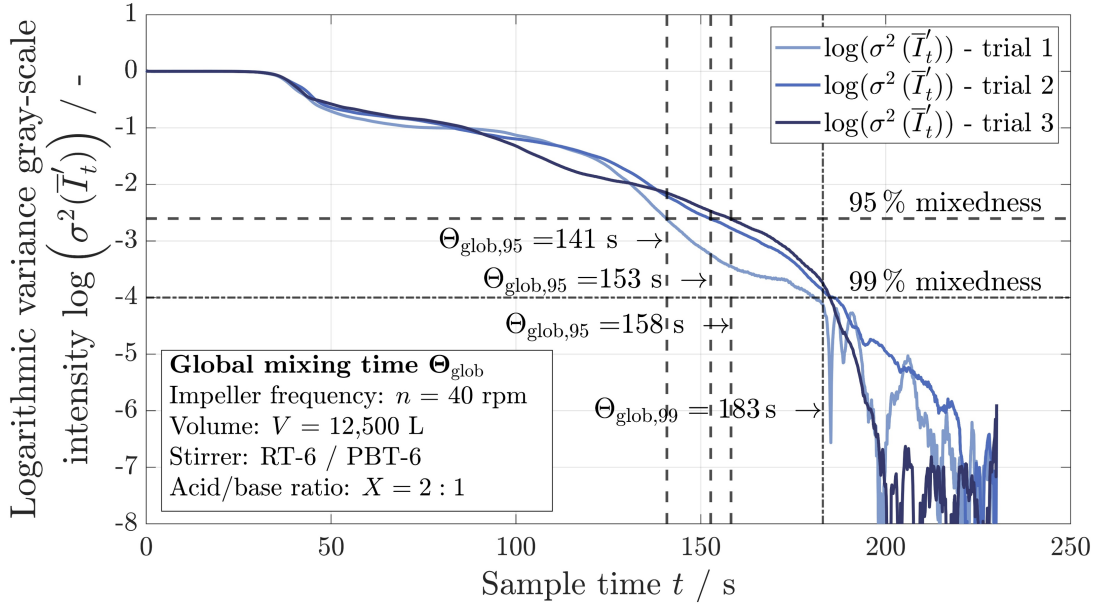


Figure 2.2: Exemplary temporal trends of the logarithmic variance of the average image gray-scale intensity from three recorded mixing time trials conducted in an STR with $V = 12,500$ L, equipped with a Rushton turbine (RT-6) and a pitched blade turbine (PBT-6). Two global mixing time criteria, such as 95 % and 99 % mixedness (horizontal lines), are indicated, along with the corresponding global mixing times Θ_{glob} (vertical lines) for each trial. An acid/base ratio of $X = 2 : 1$ is used. An acid/base ratio of $X = 2 : 1$ was applied.

the normalized average gray-scale intensity over time according to Paul et al. [Pau04] as

$$\bar{I}'_t = \frac{\bar{I}_t - \bar{I}_0}{\bar{I}_\infty - \bar{I}_0}. \quad (2.1)$$

Considering that the overall decay of concentration fluctuations follows an exponential trend [Cor57], using the logarithm of the variance of the previously calculated normalized average gray-scale intensity $\bar{I}'_t$ as a function of the sample time t according to

$$\log(\sigma^2(\bar{I}'_t)) = \log(\bar{I}'_t - 1)^2 \quad (2.2)$$

provides a more accurate estimate of the eventual mixing time with its predefined criterion. Figure 2.2 shows three trends for independent mixing time trials and their respective 95 % global mixing time $\Theta_{\text{glob},95}$ and 99 % global mixing time $\Theta_{\text{glob},99}$ for the aforementioned STR equipped with a 6-bladed Rushton turbine (RT-6) and a 6-bladed pitched blade turbine (PBT-6).

Local mixing time distribution

In contrast, the local mixing time considers spatially resolved local values individually across the reactor and the respectively measured final state of mixedness. Although this measurement evaluation requires a much higher computational power due to a pixel-wise resolution, it enables identifying aforementioned poorly mixed areas in the reactor and provides insights into hydrodynamic gradients in form of compartments [Fit21].

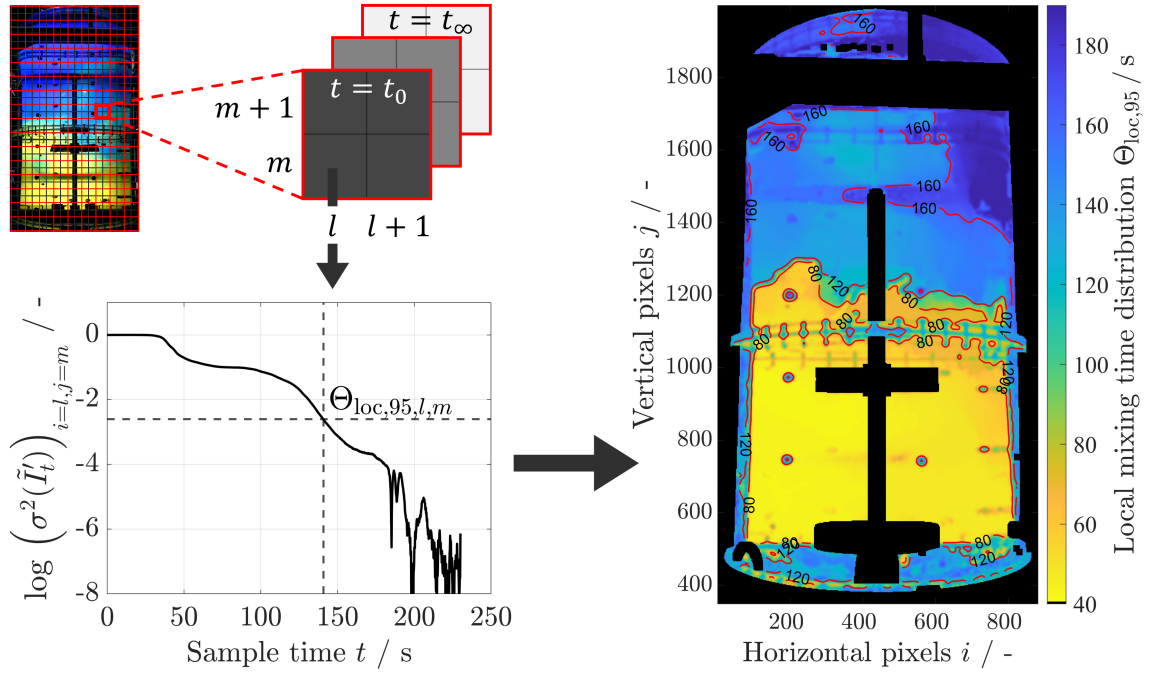


Figure 2.3: Exemplary working principle of a local mixing time distribution evaluation with the 95 % criterion for the 12,500 L STR, equipped with a Rushton turbine and a pitched blade turbine. In a 2×2 pixel array, the local mixing time $\Theta_{\text{loc},95}$ is calculated in one pixel $\{i = l, j = m\}$ based on the normalized gray-scale intensity progression $\tilde{I}'_{t,l,m}$. This value is subsequently taken for the respective array and eventually yields a local mixing time distribution throughout the reactor after applying this approach over all pixel arrays in the image.

Figure 2.3 illustrates the working principle of the evaluation of a local mixing time distribution utilized in this thesis. To make the analysis more efficient and time-effective, the normalized gray-scale intensity over time of each 2×2 pixel array $\tilde{I}'_t$ in the pixels $\{(l, m), (l+1, m), (l, m+1), (l+1, m+1)\}$ can be approximated by utilizing only the temporal values of one pixel $\tilde{I}'_{t,i=l,j=m}$ according to

$$\tilde{I}'_{t,(l,m),(l+1,m),(l,m+1),(l+1,m+1)} = \tilde{I}'_{t,l,m} = \frac{\tilde{I}_{t,l,m} - \tilde{I}_{0,l,m}}{\tilde{I}_{\infty,l,m} - \tilde{I}_{0,l,m}}. \quad (2.3)$$

Theoretical background

Hence, the calculated local mixing time is used for the entire pixel array. Ultimately, applying this approach to all pixel arrays in the image yields the local mixing time distribution throughout the 2D projection of the STR. Hence, the computational time is only a fourth and contains sufficient information about hydrodynamic gradients on the macro-scale.

While the analysis of local mixing times based on 2D projections already provides essential insights into macroscopic mixing heterogeneities and large-scale compartmentalization within a bioreactor, such an approach relies on certain simplifications. Assumptions regarding depth-averaged mixing behavior, meaning homogeneity along the optical axis, limit the resolution of three-dimensional (3D) transport phenomena. In order to resolve these limitations and capture the full complexity of fluid motion including local velocity information, the perspective of a particle inside the fluid flow needs to be scrutinized. By focusing on particle trajectories in 3D space, a more detailed understanding of unsteady flow fields and micro-scale mechanisms can be gained, which are essential to unveil phenomena relevant for biological processes.

2.2 Experimental particle tracking on laboratory scale

This work focuses on the fluid flow in stirred tank reactors (STRs) of different sizes and the behavior of applied particles to investigate the resulting flow structures. In order to understand and classify the results, this section states briefly the progress made in laboratory particle tracking in recent years, a state-of-the-art tracking methodology, and its advances in comparison to other approaches.

Flow field measurement techniques in transparent media

It is of great interest to understand unsteady flow fields and, in particular, particle-laden flows of finite-sized particles. In the natural environment, pathogens are transported through the air by atmospheric currents [Bro02], clouds are made up of water droplets that are suspended in turbulent airflows [Sha03], and oceans play a critical role in the circulation of nutrients, algae, and other microorganisms across the globe [Tho05]. In all disciplines, particles in the flow are measured to investigate the fluid. Depending on the research question and measurement technique, the particles used have a specific size, shape, and density. In the case of a transparent fluid without natural tracers and a low disperse phase fraction, either selective or imaging measurement methods can be applied [Sch11].

An example for a selective measurement method is Laser Doppler Anemometry (LDA). By means of two or more coherent intersecting laser beams (monochromatic laser light),

2.2 Experimental particle tracking on laboratory scale

an interference fringe pattern is created in the measurement volume. As tracer particles pass through this region, they scatter light that is received by a photodetector, where the frequency of the scattered signal is proportional to the velocity component perpendicular to the interference fringes [Wu89, Alb03]. Although this technique measures a wide velocity range with a high accuracy [Ruc90], the two major disadvantages are the small measurement volume and the limited sampling rate in low particle seeding densities [Sch11].

In the case of imaging measurement methods, Particle Image Velocimetry (PIV) and Particle Tracking Velocimetry (PTV) in two dimensions (2D) or three dimensions (3D) have been developed and widely utilized in recent decades. In contrast to LDA, a calibration of spatial dimensions is conducted prior the measurement, using particles that are either fluorescent or backscatter light from the source.

2D-PIV enables planar velocity field measurements with one high-speed camera by calculating the displacement of particle positions between consecutive images. With utilizing two angled high-speed cameras, Stereo-PIV offers velocity vectors in all three dimensions; however, only for those illuminated particles within the thin laser sheet volume [Bel12]. Tomographic PIV (Tomo-PIV) is based on the volumetric reconstruction of particle images into discretized voxels from multiple camera views. To do so, a tomographic reconstruction algorithm (e.g. multiplicative algebraic reconstruction technique [Her76]) necessitates an accurate calibration and camera image triangulation, and eventually yields the 3D velocity vector distribution in the respective measurement volume [Els06]. In this process, particle images are correlated from two consecutive time steps by dividing the measurement volume into subvolumes (subimages) and identifying the most similar particle distribution in the following image, based on the correlation of measured intensity patterns. Although powerful, these cross-correlation based techniques average the velocity information in the respective interrogation window, and thus, they assume a statistically homogeneous tracer particle motion. As a result, they do not capture the individual trajectory of a single fluid element in the flow.

Particle Tracking Velocimetry (PTV)

In contrast, PTV identifies and tracks individual particles over time, enabling Lagrangian flow analysis. It is a valuable tool for investigating turbulent, unsteady, and 3D fluid flows [Dab19], and delivering Lagrangian acceleration statistics [LP01] as well as Lagrangian velocity statistics [Xu08], which reflect the conditions and forces acting on a fluid element or any small flow-following particle. In particular, state-of-the-art 3D Lagrangian particle tracking (LPT) techniques and algorithms [Oue06] allow particle densities of up to 0.2 particles per pixel (ppp) by applying the "Shake-The-Box" (STB) algorithm [Jah21, Sch16]. STB, which is used

Theoretical background

in this work and presented in Chapter 4, was developed to maximally exploit both spatial and temporal information in particle tracking. The algorithm integrates four key advancements: the volumetric self-calibration (VSC) [Wie08], the optical transfer function (OTF) [Sch13], the iterative particle reconstruction (IPR) algorithm [Wie13], and the prediction step [Sch16], where the latter estimates the new particle distribution in the next time step combined with the subsequent shaking procedure to match intensity values across the different cameras (image matching or shaking).

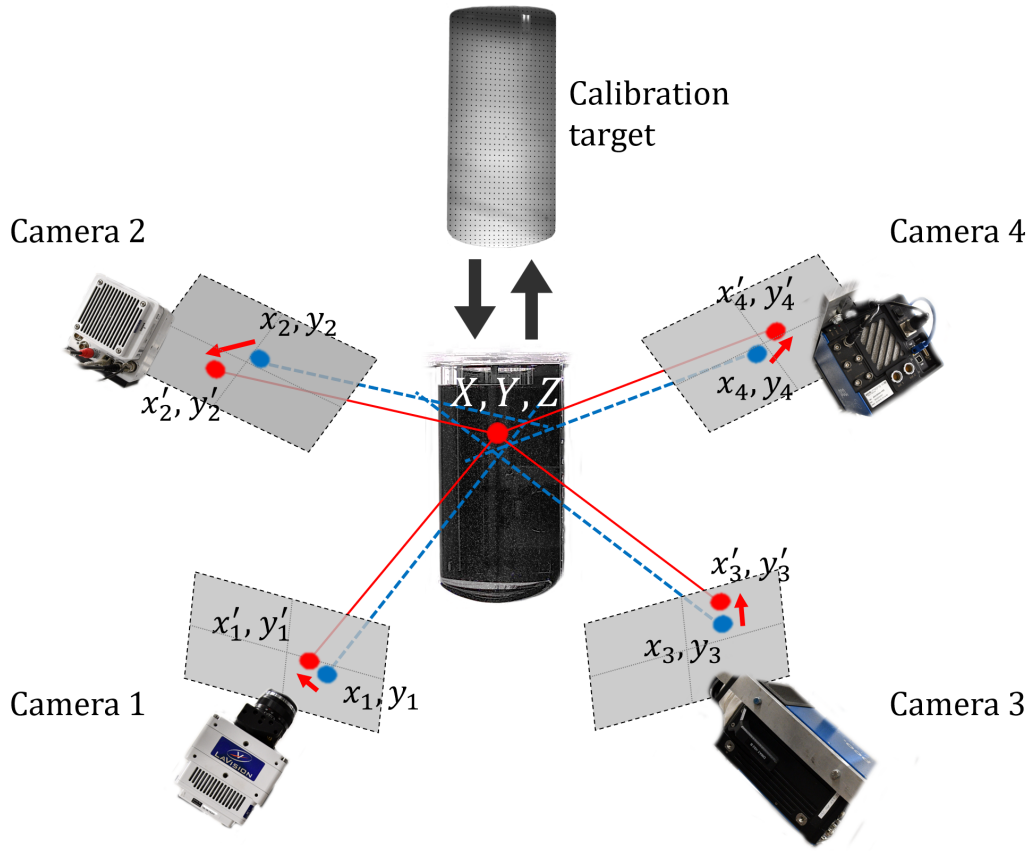


Figure 2.4: Exemplary depiction of a 4D-Particle Tracking Velocimetry (4D-PTV) setup with four cameras, a calibration target and a stirred tank reactor with incorporated particles. Each camera has an initial particle projection on its sensor (blue dot). After a successful particle triangulation with all cameras, the particle image is back-projected onto the sensor (red dot). The resulting particle shift (triangulation error) creates a single disparity vector (red arrow).

Figure 2.4 illustrates the working principle of the calibration step, the VSC, and the resulting disparity vector corrections in a 4D-PTV setup with i cameras (here: $i = 4$) as utilized in Chapter 4. The initial calibration with the calibration target yields, for each camera i , a

2.2 Experimental particle tracking on laboratory scale

mapping function $M(X, Y, Z)$ from world coordinates to camera image coordinates (x, y) as

$$(x_i, y_i) = M_i(X, Y, Z). \quad (2.4)$$

However, such target calibrations are prone to errors arising from minimal displacements, mechanical vibrations, or physical interaction, which are causing a high degree of ghost particles and noisy signals. To mitigate these issues, the subsequent VSC refines the calibration by utilizing the experimental particle images themselves [Wie08]. In this step, 2D particle positions (blue dots) are used to find corresponding 3D particle positions by matching and triangulation [Wie17], even though the lines-of-sight from all cameras do not intersect necessarily (blue dashed lines) in a single point (X, Y, Z) . Therefore, the particle position is adjusted in object space (world coordinates) and back-projected onto the respective camera sensors (red dots) with corrected particle position coordinates (x'_i, y'_i) . As a result, the final real world particle position is found by a best joint fit by means of minimizing the sum of all distances $|(x'_i, y'_i) - (x_i, y_i)|$ [Har97, Wie17]. The resulting single disparity vector $\mathbf{d}$ (red arrow) is created for each particle and camera according to

$$\mathbf{d}_i = (x'_i, y'_i) - (x_i, y_i). \quad (2.5)$$

Once sufficient particle information is available, the new mapping function M' for each camera i is provided separately as

$$M'_i(X, Y, Z) = M_i(X, Y, Z) - \mathbf{d}_i(X, Y, Z), \quad (2.6)$$

with $\mathbf{d}_i(X, Y, Z)$ being the most probable 3D disparity vector from averaged differences over many particles, in each defined (sub-)volume, in each z-plane (depth), for each camera, depending on the number of images, number of particles, threshold of particle recognition, allowed triangulation error, and number of iterations [Wie08, Wie17]. As a result, disparity vectors are constructed to correct calibration errors. Afterwards, the OTF delivers a correction of the particle intensity and shape from particle images to diminish effects of optical distortions and limited sharpness to further refine the calibration [Sch13, Sch23a].

As depicted in Figure 2.5, after a successful system calibration, 2D particle image peaks are detected in each camera view for each time step (red dots) and are subsequently used to reconstruct the corresponding 3D particle positions, if the lines-of-sight from several cameras intersect within a preset triangulation error (red lines, Fig. 2.5 a)). In this complex and iterative IPR algorithm, particle peaks are detected, triangulated, positions and intensities optimized, ghost particles filtered and residual images rendered [Wie13, Wie17, Jah21].

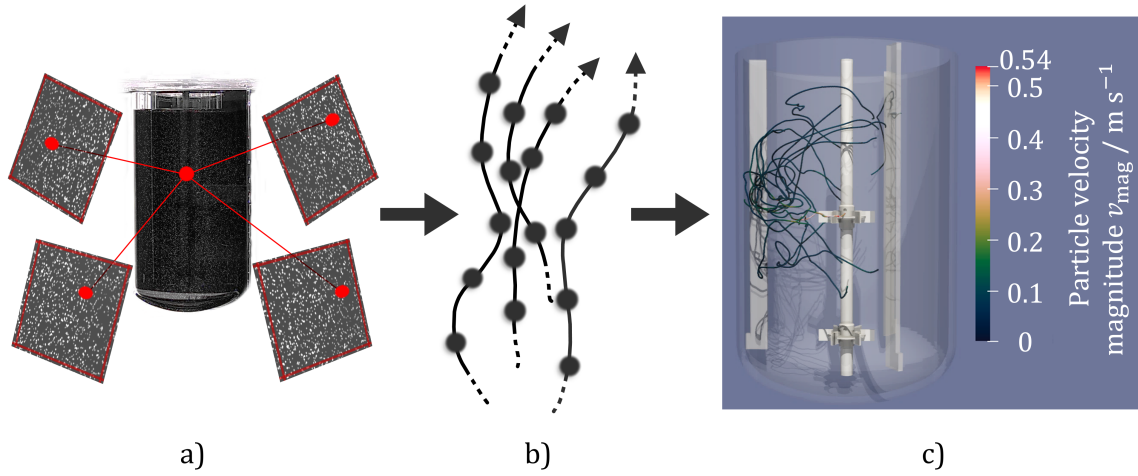


Figure 2.5: Working principle of a Lagrangian particle tracking experiment: a) Determination of particle image peaks in 2D camera images and calculating 3D particle positions by means of an iterative particle reconstruction algorithm including triangulation; b) Temporal connection of particle clouds to singular trajectories by means of the Shake-the-Box algorithm; c) Post-processing of results for e.g. Lagrangian velocities; according to Schröder and Schanz [Sch23a].

Thereafter, a 3D particle cloud is created and utilized to apply the STB tracking algorithm over consecutive time steps to form particle trajectories (Fig. 2.5 b)) [Wie13, Jah21, Sch23a]. In contrary to previous approaches, STB makes use of temporal information and estimates the particle distribution of the subsequent time step based on existing particle tracks [Sch16]. Hence, future particle positions are predicted by extrapolating previously established trajectories and serve as an initial guess for an extended IPR procedure. This technique eliminates the necessity for triangulation for the majority of particles in the measurement volume and is therefore computationally more efficient than Tomo-PIV, which requires triangulating each bright dot on every camera at every time step. It also enables the efficient reconstruction of 3D particle fields at high seeding densities while maintaining high tracking accuracy and minimizing the occurrence of ghost particles. Eventually, 3D Lagrangian particle tracks can be post-processed (Fig. 2.5 c)), allowing highly spatially resolved fluid dynamic flow data to be obtained.

The ability to accurately reconstruct and evaluate 3D Lagrangian particle tracks in laboratory-scale flows represents a significant advancement in capturing high-resolution fluid dynamic data. However, in industrial bioprocesses, the primary challenge lies in detecting and characterizing spatial and temporal heterogeneities at much larger scales in opaque stainless steel reactors. To achieve this, advanced sensor technologies capable of measuring process parameters and relevant gradients within industrial reactors are required. The development

2.3 Advancements in mobile sensor devices with a focus on biotechnological applications

and application of such mobile sensor devices, often referred to as "Lagrangian Sensor Particles" [Hof22b, Pör23, Hof24b, Hof25f], represents a promising approach to bridge this gap, enabling a deeper understanding of large-scale process heterogeneity and its impact on biotechnological production.

2.3 Advancements in mobile sensor devices with a focus on biotechnological applications

Parts of this section are based on the third chapter "Advances in Characterization of Industrial Bioreactors for Cell Culture Process" in the book "Biopharmaceutical Manufacturing - Progress, Trends and Challenges" by Fitschen, Hofmann, Kursula, Haase, Wucherpfennig and Schlüter [Pör23].

The history of automated measuring devices and their information transmission from one device toward another dates back to the inventors Tesla, Marconi, Braun, and Maxwell [Bra09, Mar01, Max65, Tes00]. Eventually, their inventions led to the first radio remote control system in 1938, which was able to generate and modulate a radio signal wave for controlling a radio apparatus at a remote point [Koc41]. Several decades later, in 1996, Intel, IBM, Ericsson, Nokia, and other researchers began developing an industry standard for a close-range radio link. As part of this wireless initiative, Jim Kardach proposed the name "Bluetooth" in 1997, drawing an analogy to the Danish king Harald "Bluetooth" Gormsson, who united the tribes of Denmark, much like the technology was intended to unite devices [Kar25, Ada25]. While measuring capabilities, transmitting distance, functionality of components, and reliability increased in the upcoming years, sensor sizes and its production costs decreased rapidly. In the entertainment industry, not only would people communicate with each other, but also with wireless accessories such as laptops, TVs, headphones, cars, and even light bulbs. The Internet of Things (IoT) was born. A technology in which objects equipped with sensors, actuators, and processors communicate with each other to create context awareness [Set17].

Overview of sensor applications in multiple industrial sectors

According to the Google Android developer documentation, the most commonly used sensors are classified into three main categories: motion sensors, position sensors, and environment sensors [And25]. Motion sensors such as an accelerometer and a gyroscope monitor the movement of the device. Position sensors measure the physical position of the device, such

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as the Global-Positioning System (GPS), magnetometer, or a proximity sensor. Environment sensors return a single value for each data event, such as pressure, temperature, humidity, light, or touch.

In the automotive industry, early innovations laid the groundwork for wireless sensing of parameters such as pressure, temperature, imbalance, or other values [Fri72]. Today, more than 50 sensors are installed in a middle-class car [Sko20], and even more in an autonomous vehicle, which is understood to be classified at Level 3, 4 or 5 according to driving automation standards [SAE25]. However, the main focus here is on constant location determination for the respective vehicle by means of exteroceptive (perceiving the environment) and proprioceptive (measure values within the system) sensors. The first can be light detection and ranging (LiDaR), radio detection and ranging (RaDaR), camera, and ultrasonic. The latter are GPS, the Inertial Measurement Unit (IMU) and Encoders [Cam18].

In healthcare, wireless sensors were already used in the 1950s in veterinary medicine to perform a respiratory study on dogs [Mor53]. Several years later in a human study, a cylindrically shaped deglutible pill with a total length of 25 mm to measure the pressure in the gastrointestinal tract wirelessly [Con60, Far58]. Today, patients are monitored in real time [Val17, Nat25] and the idea of a fully implantable subcutaneous sensor [Mor14] or an ingestible sensor has been further developed [Zde11]. Eventually, it received approval as a medical device from the U.S. Food & Drug Administration (FDA) [Pro20b, Pro20a].

The evolution of "radio pills" within the healthcare sector, as presented by Rowlands and Wolff in 1961 [Row61], and subsequently by Watson et al. in 1962 [Wat62], led to a design with the size of approx. 20 mm and the application in 1965 to evaluate internal stresses of sand at rest and flowing in bunkers [Han65] (Fig. 2.6 a)). In their work, an altering pressure in the environment forced upon a pressure diaphragm, which resulted in an instant change in inductance via installed inductive coils and thus in a frequency variation of the transmitted signal. This integration of a transducer with a transmitter in an encapsulated housing marked one of the first uses of wireless free-floating sensor particles in the manufacturing industry [Pör23]. Most recently, a novel wireless and passive magneto-mechanical resonator (MMR) sensor has been developed to track spatial position and orientation and to measure physical quantities like pressure and temperature, originally for medical applications but with high potential for process engineering. Tracking works by measuring the sensor's response to a weak external magnetic field. Sensing works by detecting changes in its resonance frequency, which depend on the distance between internal magnets and can be linked to mechanical quantities. This allows wireless measurements even in inaccessible environments. This technology offers advantages such as battery-free operation, cost-effective production, and improved localization in complex media [Gle23, Kno24, Mer25].

2.3 Advancements in mobile sensor devices with a focus on biotechnological applications

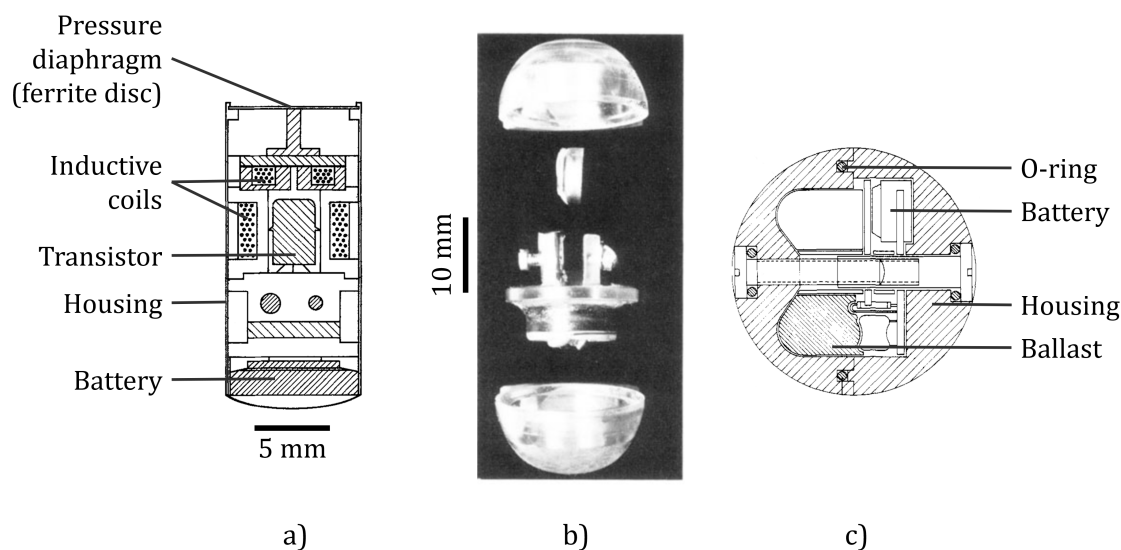


Figure 2.6: a) Cross-section of "radio pill" according to Handley and Perry [Han65]; b) exploded view of "flow follower" according to Mitchell [Mit69]; c) cross section of "radio pill" according to van Barneveld et al. [VB87a].

In biotechnological processes, various sensors are widely implemented to monitor fermentation parameters such as temperature, pH, pressure, chemical composition, cell density, and conductivity, among others [Bus17]. Stationary *in situ* probes, placed at fixed positions, are typically used to obtain the data, either at a single point (e.g. a probe belt in the reactor bottom) or distributed radially or axially along the reactor wall [Gar20]. However, these sensors provide time-resolved but spatially limited information, which can result in undetected spatial gradients throughout the reactor and eventually reduce product quality and quantity. Moreover, due to their fixed position, they are unable to track how long cells are exposed to specific metabolic conditions in occurring compartments. As an alternative, mobile sensors in the form of particles suspended in the fluid can move with the flow according to their physical properties, recording process data along their trajectories, and thus offering insights into the dynamic environment within the reactor. Since these particles collect critical Lagrangian data along their path and essentially mimic the lifeline of a microorganism during cultivation, they are referred to as "Lagrangian Sensor Particles" (LSP) [Hof22b, Pör23, Hof24b, Hof25f]. Initial efforts to quantify circulation times within a bioreactor during a fermentation were made in 1969. This was achieved using a hollow sphere equipped with a radio transmitter operating at a carrier frequency of approximately 2 MHz, coupled with a 35 mAh mercury battery, with a total diameter of 20 mm (Fig. 2.6 b)). The evaluations were carried out successfully in stirred tank reactors (STR) with a capacity of 450 L [Mit69]. Subsequent

Theoretical background

developments were achieved to measure the circulation times in aerated STRs (Fig. 2.6 c)) [Bry79, Mid79, VB87b, VB87a].

Recent developments of Lagrangian Sensor Particles in biotechnology

In recent years, multiple research teams have published and discussed their LSP designs, of which six intended for biotechnological applications are briefly presented in terms of their progress and sensor equipment (Fig. 2.7 and Tab. 2.1).

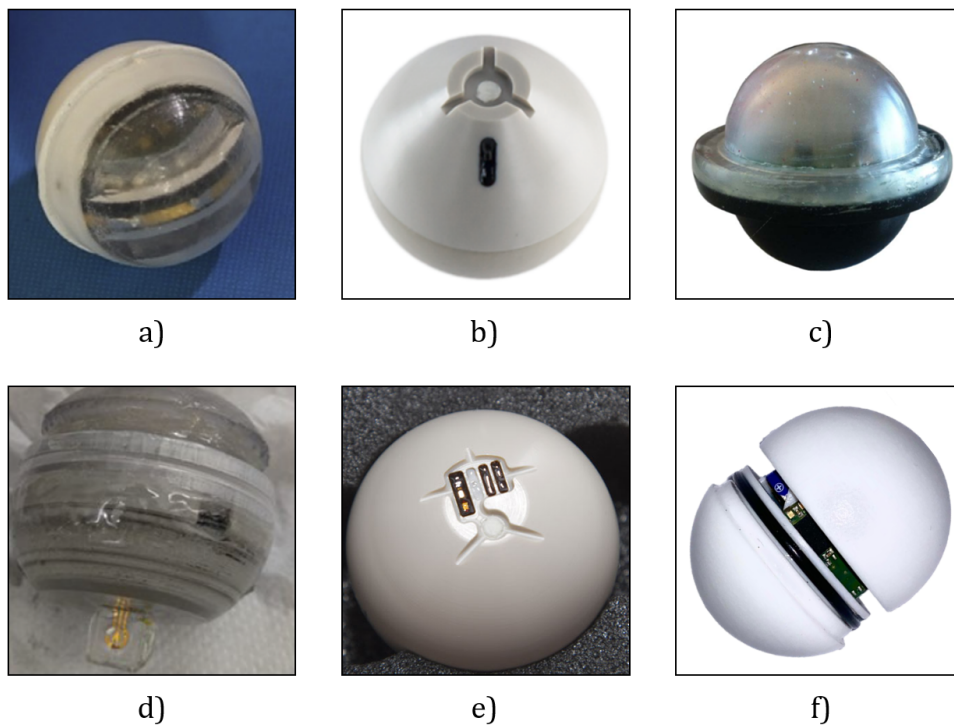


Figure 2.7: Lagrangian Sensor Particle developers: a) “Sens-o-Sphere” by Lauterbach et al. (amensio & Technische Universität Dresden, Germany); b) “smartCAPS” by Zimmermann et al. (former smartINST SAS, France); c) Todtenberg et al. (Innovations for High Performance Microelectronics & Fraunhofer Institute for Biomedical Engineering, Germany); d) “bPod” by Stine et al. (University of Maryland, USA); e) “FermSense 3D” by Bisgaard et al. (former Freesense ApS, Denmark); f) Buntkiel et al. (Helmholtz-Zentrum Dresden-Rossendorf, Germany) [Pör23].

- a) Lauterbach et al. manufacture the smallest LSP designed for biotechnological purpose, called "sens-o-spheres". The published design measures only 8.9 mm (formerly 7.9 mm) in diameter and weighs approximately 0.5 g. The LSPs are capable of being recharged inductively and are equipped with an antenna that transmits recorded

2.3 Advancements in mobile sensor devices with a focus on biotechnological applications

Table 2.1: Overview of LSP developer teams, their LSP size d_{LSP} , and utilized sensors such as temperature (T), pressure (p), pH value (pH), conductivity (C), dissolved gas (c_{gas}), Inertial Measurement Unit (IMU) and further installed sensors. Asterisk (*): no peer-reviewed data found.

Developer	Diameter / mm	T	p	pH	C	c_{gas}	IMU	Further sensors
a)	$7.9 \leq d_{LSP} \leq 8.9$	✓						–
b)	25	✓	✓	✓	✓			Acceleration
c)	44			✓	✓			Concentration of potassium, sodium, and glucose
d)	60					✓		–
e)	$45 \leq d_{LSP} \leq 55$	✓	✓	✓		✓*	✓	–
f)	$40 \leq d_{LSP} \leq 90$	✓	✓		✓		✓	Ultra-wide-band module

temperature data at a maximum frequency of 0.1 Hz, using a carrier frequency of 433 MHz, to a receiver positioned outside the STR. A maximum of 24 LSPs can be operated simultaneously [ame25, Lau19b]. Experimental studies showed a successful implementation in laboratory shake flasks [Lau19a], as well as in a 200 L and a 2000 L single-use bioreactor filled with deionized water (not published).

- b) The former "smartINST" research team also conducted *in situ* wireless measurements (via 433 MHz) utilizing different types of LSPs, all with a diameter of approximately 25 mm. With a weight of approximately 8 g the LSPs were autoclavable, deployable in an ATEX environment, and capable of recording up to 500 days with a respectively adjusted sampling rate [sma20]. The publication of Zimmermann et al. stated that the LSPs were equipped with either a temperature, pH, conductivity, or pressure sensor in a polyether ether ketone (PEEK) housing [Zim13a, Zim13b]. To the best of the author's knowledge, there has been no further progress or advancements in this development since 2015.
- c) The third team around Todtenberg et al. aimed for developing an LSP to allow remote observation of algae culture growth in a photobioreactor. With a carrier frequency of

Theoretical background

433 MHz and utilizing potentiometric, amperometric, and conductivity sensors, the initial LSP prototype exhibited a packet error rate of 26 % while traversing through the reactor in nutrient solution. Despite achieving a successful signal processing chain for the potentiometric sensor values in different pH buffer solutions in the second prototype, the development was not continued since 2015, to the best of the author's knowledge [Tod13b, Tod15].

- d) Stine et al. presented an innovative *in situ* dissolved oxygen measurement LSP with the name "bPod". Measuring 60 mm in diameter, it is equipped with a potentiostat analog-front-end paired with a Bluetooth low energy microcontroller for processing the electrochemical Clark-type sensor signal. The system was successfully tested in a 10 L bioreactor [Sti20a, Sti20b]. Meanwhile, the research team focuses on miniaturized ingestible gas-sensing (H_2S) devices for a minimally invasive diagnosis in the gastrointestinal tract [Sti22, Sti24], similar to the "Atmo Capsule" (Atmo Bioscience, Australia), which is also an ingestible disposable gas-sensing (O_2 , H_2 , CO_2) capsule to treat bowel syndromes [Zho24, Atm25].
- e) Freesense ApS, established in 2015, originated from technology developed at the Technical University of Denmark. Three LSP sizes were published, of which one measured approximately 43 mm and the other two 52 mm and 55 mm in diameter. The initial design, called "FermSense 3D" integrated an ISFET pH sensor, alongside a temperature and a pressure sensor, as well as an IMU [Bis20]. Later, the second design "FermSense DO" replaced the pH sensor with a dissolved oxygen (DO) sensor; however, no peer-reviewed publications are found for this design, which is denoted accordingly in Table 2.1. Besides circulation time measurements in a 580 L STR [Bis21b, Bis21a], the first design was also deployed in a 600 m³ bubble column reactor to track temperature and pressure. Collected pressure data was correlated with the axial position, and contributed to creating an automated compartment model [Bis22]. In 2022, Freesense ApS decided to discontinue their startup. Nonetheless, very recently, Haringa et al. demonstrated that FermSense 3D data ($d_{\text{LSP}} = 52$ mm, measuring pressure, pH and temperature) can be utilized to partly validate computational fluid dynamics (CFD) simulations (ANSYS FLUENT 2020R1) in a 2 m³ bubble column [Har24].
- f) In 2009, the research team from the Helmholtz-Zentrum Dresden-Rossendorf presented their first LSP design with the intended use in stirred fermentation biogas reactors. The first designs by Thiele et al. were cylindrically shaped, with a height of approximately 85 mm and a diameter of 45 mm, and equipped with an NTC thermistor, a pressure

2.4 Lattice-Boltzmann large eddy simulations for particle-laden flows

sensor and an accelerometer [Thi09, Thi10, Rei13]. Further on, the LSP was extended with a buoyancy control unit (via an installed piston) and a magnetometer to improve circulation time measurements in bioreactors and fermentors on laboratory scale [Rei16, Rei15]. Today, the most recent designs are spherical with different diameters between 40 mm and 90 mm, depending on their application and sensor equipment. Additional sensors such as an IMU (9-degree of freedom) [Bun23], an ultra-wide-band communication unit, or a conductivity sensor [Bun21] were added to a state-of-the-art developed LSP platform. This thesis makes use of the 40 mm LSP design, which is equipped with a pressure sensor, an IMU, and a temperature sensor [Bun23, Hof24b, Hof25f].

As presented, LSPs can be utilized in different sizes and types of STRs as well as in bubble columns, and biogas digesters with high-viscous fluids. In addition, their application in pipelines [Dui20, Dui18], as environmental sensor devices [Pfl19, Den19, AIO21], to characterize turbulent free-surface flows [Cab21], or to investigate the gastrointestinal track [Caf15] has contributed significantly to the understanding of the respective system. Consequently, they offer valuable experimental insight; however, they cannot yet fully resolve the complex micro-scale hydrodynamics of industrial-scale bioprocesses on their own. This limitation highlights the necessity of computational approaches to complement experimental observations. CFD serves as a powerful tool to simulate fluid flow and particle transport across scales, and enables deeper apprehension of spatiotemporal flow. In this context, LSP technology additionally provides crucial information on particle transport, along with hydrodynamic characterization data to validate numerical simulations and improve the understanding of evolving heterogeneities.

2.4 Lattice-Boltzmann large eddy simulations for particle-laden flows

In the biopharmaceutical industry, computational fluid dynamics (CFD) is widely used for complex numerical simulations of fluid flow fields and particle flow in various unit operations. In doing so, assessments of new process designs, process improvements in up- and downstream development, and troubleshooting are meanwhile conducted on a day-to-day basis. This work utilizes Lattice-Boltzmann large eddy simulations (LB LES) carried out in the propriety software Dotmatics M-Star CFD, LLC. (Versions 3.0.19 and 3.10.32). Therefore, in this section, a brief overview of different CFD model approaches is given,

Theoretical background

the underlying physics and assumptions for the herein used simulations are outlined, and different simulated particle types are presented.

In CFD, a continuous fluid mechanics problem can be discretized into smaller sections using a "mesh". Each mesh is allocated to a discrete variable such as pressure p or velocity u , and must satisfy the continuity equation (mass), the energy equation (heat), and the Navier-Stokes equations (momentum) with their respective boundary conditions.

Due to challenges such as nonlinearity and complex geometries, numerical methods such as finite difference, finite volume, and finite element techniques are employed. Transformation of these partial differential equations into algebraic equations throughout the mesh and solving them necessitates high computational resources due to a large number of repetitive and iterative calculations until convergence [Fox10]. In this approach, the fluid is treated as a continuum with its conservation laws and is thus described on a macroscopic scale [Guo13, Bat00, Moh19].

On a microscopic scale, the medium is considered with regard to all its containing molecules (or atoms), where each obtains a position and velocity. In this case, the determination of intermolecular forces and the application of Newton's second law of motion is utilized, which entails momentum conservation, to obtain the position and velocity of each molecule over time. This molecular dynamics (MD) simulation is utilized to model the behavior on an atomic scale, which is the reason why its applicability is still limited to sub-micrometer scales because of the large number of constituent particles with $N_A = 6.022 \cdot 10^{23} \text{ mol}^{-1}$ [Moh19, Guo13].

The Boltzmann equation

A compromise between both previously mentioned approaches states the Boltzmann method, which accounts for the space-time dynamics of a particle ensemble by representing it as a distribution function. On this mesoscopic scale, there is no need to solve the Navier-Stokes equation, but instead solving the Boltzmann equation

$$\frac{\partial f}{\partial t} + \boldsymbol{\zeta} \cdot \nabla_x f = \Omega(f), \quad (2.7)$$

which describes the transportation of the particle distribution function f [Guo13] without any external force $\mathbf{F}_{\text{ext}}$. $\boldsymbol{\zeta}$ denotes the particle velocity vector. Ω is the collision operator, describing the rate of change from the initial to the final status of the velocity distribution function. It conserves mass, momentum and energy [Guo13].

2.4 Lattice-Boltzmann large eddy simulations for particle-laden flows

Due to its complexity, in 1954, Bhatnagar, Gross, and Krook (BGK) [Bha54] proposed a simplified model, where the collision parameter can be substituted by

$$\Omega(f) = \frac{1}{\tau} (f^{\text{eq}} - f). \quad (2.8)$$

τ constitutes the relaxation factor (time to reach equilibrium state), and f^{eq} is the local equilibrium particle distribution function (or Maxwell–Boltzmann distribution function) so that the entire equation can be written as

$$\frac{\partial f}{\partial t} + \boldsymbol{\zeta} \cdot \nabla_x f = \frac{1}{\tau} (f^{\text{eq}} - f), \quad (2.9)$$

which replaces the Navier-Stokes equation in CFD and is also called *lattice* BGK (LBGK) in literature [Guo13]. The left-hand side of the LBGK expresses the advection (or streaming), whereas the right-hand side denotes the collision (or source term) [Moh19]. In the next step, the LBGK is discretized so that a finite number of modeled velocity vectors is assigned to grid points in a determined mesh. Qian et al. proposed multiple α -dimensional β -velocity models ($D_\alpha Q_\beta$) [Qia92], of which D3Q19 is utilized in this thesis (Fig. 2.8). In this case, the Navier-Stokes equations can be obtained by means of the Chapman-Enskog expansion method [Guo13]. Additionally, by applying the forward Euler expansion to each of these individual discrete velocity vectors derived from Equation 2.9 in the D3Q19 structure, and subsequently performing a normalization, the equation

$$f_i(\mathbf{x} + \mathbf{c}_i \Delta t, t + \Delta t) = f_i(\mathbf{x}, t) + \Delta t \Omega_i(\mathbf{x}, t) \quad (2.10)$$

is obtained, with $\mathbf{c}_i = \boldsymbol{\zeta}_i \cdot 3^{-1/2}$, denoting the discrete lattice velocity [Krü17].

Coupling of Lattice-Boltzmann with large eddy simulations

For resolving turbulent flows, three numerical methods are presented in the literature, which are direct numerical simulation (DNS), Reynolds-averaged Navier-Stokes (RANS), and large eddy simulation (LES) [Guo13]. DNS provides a direct numerical solution of the Navier-Stokes equations without relying on turbulence models, resolves all scales of motion directly with a high spatial and temporal resolution (from the smallest dissipative scale up to the integral scale) and thus requires high computational demands. RANS focuses more on the combination of mean flow characteristics and the corresponding fluctuation on the macro-scale and applying turbulence models such as the $k - \varepsilon$ model [Suc95]. It is proved to be a highly efficient method for accurately predicting power numbers [Cor11] or mean

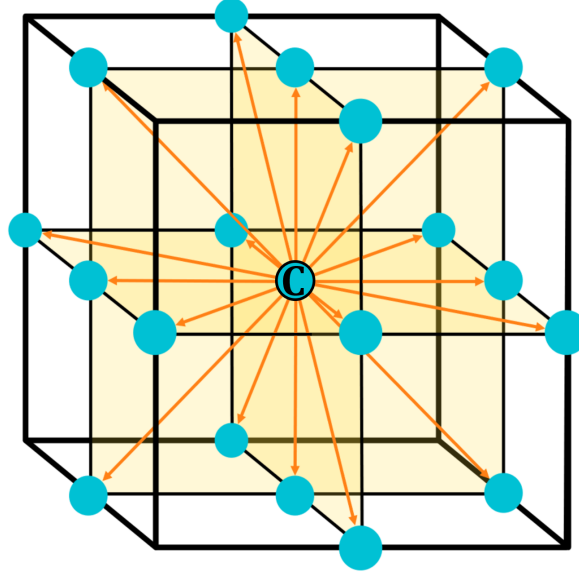


Figure 2.8: Discretization of velocity vectors pointing from the center lattice point (C) to all 18 neighbors in a D3Q19 Lattice-Boltzmann model structure [Zhu11].

flow quantities in stirred tank reactors (STR) [Bru98a]. However, in recent studies, it was outperformed by LES, which is able to successfully obtain time-resolved effects of turbulence, unsteady flow fields and, in particular, transient flow structures in terms of mixing assessment in STRs [Har18b, Har18c]. LES only addresses large-scale flow structures utilizing a spatial filter after which the Navier-Stokes equations are solved. The small-scale dynamics remain unresolved and must therefore be represented by sub-grid scale (SGS) models. A widely adopted approach is the Smagorinsky–Lilly SGS model [Sma63], which introduces a sub-grid eddy viscosity ν_t of the form

$$\nu_t = (C_S \Delta x)^2 \cdot \sqrt{2 \cdot \bar{S} : \bar{S}}, \quad (2.11)$$

where C_S is the Smagorinsky coefficient, Δx is the local lattice spacing (filter width), and $\bar{S}$ is the resolved rate-of-strain tensor.

Since the Lattice-Boltzmann (LB) method is ideally suited for parallel processing, given by its nature of localized operations in the grid (streaming and collision steps), recent rapid developments of graphics processing units (GPU) enhance computational throughput [Tho21]. Hence, coupling LB with LES is a promising possibility to predict nonlinear interactions of fluid flow structures over a wide range of scales within a reasonable amount of computation time. Moreover, it facilitates the integration of particle-based simulations, enabling accurate modeling of particle-fluid interactions [Kus21, Hof22b].

Governing equations for numerical simulation of particle dynamics

Fluid flow arises once a pressure or velocity gradient is applied to a fluid. The dynamic behavior of this motion is governed by Newton's second law of motion [New87] serving as the fundamental principle and is described as

$$\sum \mathbf{F} = m \cdot \frac{D\mathbf{u}}{Dt}, \quad (2.12)$$

where the sum of all forces $\mathbf{F}$ applied on a fluid element are equal to the product of its mass m and acceleration $D\mathbf{u} (Dt)^{-1}$. In this case, the total time derivative $D (Dt)^{-1}$ is used to describe the rate of change of a fluid element [Max83] while moving through space and time, and can also be expressed as

$$\frac{D}{Dt} = \frac{\partial}{\partial t} + (\mathbf{u} \cdot \nabla). \quad (2.13)$$

The motion of a particle suspended in a fluid is governed by a combination of forces arising from particle inertia, fluid dynamics, and their interactions. Generally, for low particle Reynolds numbers ($Re_p < 1$, Equ. 2.25), the equation of motion for a spherical particle of diameter d_p in a nonuniform, unsteady flow is proposed by Maxey and Riley [Max83] as

$$\begin{aligned} \frac{\pi}{6} d_p^3 \rho_p \frac{d\mathbf{v}}{dt} = & \underbrace{\frac{\pi}{6} d_p^3 (\rho_p - \rho_f) g}_{\text{net body force}} \\ & + \underbrace{\frac{\pi}{6} d_p^3 \rho_f \frac{D\mathbf{u}}{Dt}}_{\text{non-inertial force}} \\ & - \underbrace{\frac{\pi}{6} d_p^3 \rho_f \frac{1}{2} \frac{d}{dt} \left[\mathbf{v} - \mathbf{u} - \frac{1}{40} d_p^2 \nabla^2 \mathbf{u} \right]}_{\text{added mass force}} \\ & - \underbrace{3 \pi d_p \eta \left[\mathbf{v} - \mathbf{u} - \frac{1}{24} d_p^2 \nabla^2 \mathbf{u} \right]}_{\text{quasi-steady viscous force}} \\ & - \underbrace{\frac{3}{2} \pi d_p^2 \eta \int_0^t \frac{d}{d\tau} \left[\mathbf{v} - \mathbf{u} - \frac{1}{24} d_p^2 \nabla^2 \mathbf{u} \right] \frac{d\tau}{\sqrt{\nu(t-\tau)}}}_{\text{time history (Basset) force}} + \underbrace{\mathbf{F}_L}_{\text{lift force}}, \end{aligned} \quad (2.14)$$

provided that the particle is much smaller than the Kolmogorov length scale λ_k ($d_p \ll \lambda_k$), being the smallest eddy size (Sec. 2.5).

Theoretical background

The particle is moving with the velocity $\mathbf{v}$ in an undisturbed and incompressible fluid flow field with the velocity $\mathbf{u}$, with their respective densities ρ_p and ρ_f and the dynamic fluid viscosity η and the kinematic viscosity ν . τ represents the integration variable of the motion of the particle, and g is the gravitational acceleration. The first term on the right hand side is the particle's net body force (gravity and buoyancy). Any other term on the right hand side describes the contribution to the fluid dynamic force acting on the particle and is the non-inertial force (fluid acceleration at the particle's location, also undisturbed flow), added mass force including the Faxén correction (inertia of displaced fluid, also virtual mass term), quasi-steady viscous force including the Faxén correction (Stokes drag, also steady-state drag), the time history (Basset) force including Faxén correction (effects of past interactions, also Basset term) [Cro11], and the lift force (flow shear or rotation). The latter becomes relevant, if the particle experiences flow conditions such as Saffman lift (shear-induced) [Saf65], Magnus lift (rotation-induced) [Swa61], or other turbulence-induced forces (e.g. wall-induced), which might occur at the system boundaries [Toh21].

However, in turbulent flows ($Re_p \gg 1$) with strong and instantaneous velocity gradients, the description of particle motion becomes challenging if aiming for analytical results, in particular for finite-sized particles ($d_p \gtrsim \lambda_k$). In this regime, particles start interacting with dissipative eddies and disturb their surrounding fluid, which leads to modifications in the particle drag [Bru98b]. At high particle Reynolds numbers, the drag force becomes nonlinear (Fig. 2.14), and thus, the Stokes drag definition is no longer valid. Instead, an empirical drag law, as described in Section 2.5, must be used in the quasi-steady viscous force term. As empirical drag laws are typically fitted to experimental data, they already capture a large part of the finite-size effects of particles in a flow. The Faxén corrections, which involve Laplacian terms of the velocity field and thus account for curvature and higher-order spatial variations, can therefore often be neglected in practice. Similarly, the time history (Basset) force can be neglected due to the herein utilized particle-to-fluid density ratio of $\rho_p \rho_f^{-1} \approx 1$ [Mic97] in combination with the assumption that both the particle and the surrounding fluid have the same initial velocity [Uri25]. The lift force is assumed to have only minor statistical effects [Cal12], and is therefore neglected as well. The non-inertial force can be rewritten as the pressure gradient force, where ∇p denotes the pressure gradient vector, assuming that the pressure field is uniform over the particle's surface in an incompressible flow. Regarding the added mass term, Auton et al. suggested that the fluid velocity derivative needs to be the total time derivative (Equ. 2.13) [Aut88], and Odar and Hamilton proposed that the added mass coefficient being 0.5 can be extended by a virtual mass correction coefficient including an acceleration parameter A_c [Oda64, Mic11]. Summarizing all assumptions and

2.4 Lattice-Boltzmann large eddy simulations for particle-laden flows

adaptions, the CFD calculations utilized in this work make use of

$$\begin{aligned}
 \frac{\pi}{6} d_p^3 \rho_p \frac{d\mathbf{v}}{dt} = & \underbrace{\frac{\pi}{6} d_p^3 (\rho_p - \rho_f) g}_{\text{net body force}} \\
 & - \underbrace{\frac{\pi}{6} d_p^3 \nabla p}_{\text{pressure gradient force}} \\
 & + \underbrace{\left(2.1 - \frac{0.132}{0.12 + A_c^2} \right) \frac{\pi}{6} d_p^3 \rho_f \frac{1}{2} \left[\frac{D\mathbf{u}}{Dt} - \frac{d\mathbf{v}}{dt} \right]}_{\text{added mass force}} \\
 & + \underbrace{\frac{\pi}{8} c_D d_p^2 \rho_f |\mathbf{u} - \mathbf{v}| (\mathbf{u} - \mathbf{v})}_{\text{quasi-steady viscous force}} \\
 & + \underbrace{\mathbf{F}_p}_{\text{particle-particle interaction force}}
 \end{aligned} \tag{2.15}$$

for both inertial particles [M-S25b] and discrete element particles [M-S25a], where the acceleration parameter A_c in the virtual mass coefficient [Oda64] is given as

$$A_c = \frac{(\mathbf{u} - \mathbf{v})^2}{d_p \frac{d}{dt} (\mathbf{u} - \mathbf{v})}, \tag{2.16}$$

and the drag coefficient c_D [Bro03] is defined as

$$c_D = \frac{24}{Re_p} \left(1 + 0.15 Re_p^{0.681} \right) + \frac{0.407}{1 + \frac{8710}{Re_p}}, \tag{2.17}$$

with the particle Reynolds number Re_p (see Equ. 2.25). The particle-particle interaction force applies only for simulations of Lagrangian Sensor Particles (LSP) in Chapter 6 by means of the Hertzian contact model (e.g. Hertz simple with a specific Young's Modulus, Poisson's Ratio, etc.) [M-S25c].

Overview of simulated particle types in this work

In this work, an experimental and numerical multi-scale analysis of different types and sizes of particles is conducted in turbulent flow fields in STRs. Regarding LB LES, Figure 2.9 depicts the four types of particles used.

Theoretical background

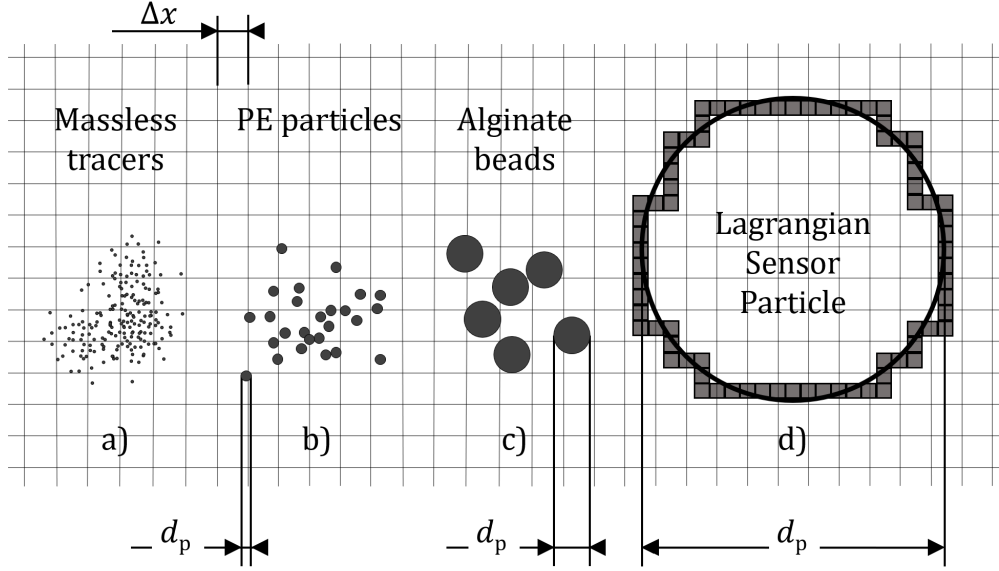


Figure 2.9: Exemplary two-dimensional depiction of the lattice fluid domain with a lattice spacing of Δx , denoting the four simulated particle types used in this work: a) non-inertial, non-interacting point objects referred to as massless tracers; b) Polyethylene (PE) particles with $d_p < \Delta x$; c) alginate beads with $d_p \approx \Delta x$; d) Lagrangian Sensor Particle with $d_p > \Delta x$, which is treated as a discrete element particle (thick circle shows a set of Lagrangian points). As a reference, the coarse computational boundary (squares) based on bounce-back rules of the LB method is demonstrated according to Feng and Michaelides, but not used in this work [Fen04].

The first particle type is the "massless tracer", the second is the "Polyethylene particle" (PE particle), the third is the "alginate bead", and the fourth is a "Lagrangian Sensor Particle". The tracers are non-inertial, non-interacting point objects perfectly following fluid streamlines, and thus, they do not possess mass [M-S25d]. PE particles and alginate beads are either smaller than the lattice spacing Δx , or approximately the same size as Δx , respectively. Both are referred to as "inertial particles", since they possess mass [M-S25b]. The LSP is considered as a "discrete element particle" with mass, for which particle-particle contact mechanics is additionally calculated [M-S25a]. The no-slip boundary condition is applied at the interface between the particle surface and the fluid.

For both Chapters 4 and 6 that involve numerical simulations, extensive mesh studies are conducted to ensure numerical independence of the results obtained in each study [Kus21, Hof25f]. Accordingly, in Chapter 4, a grid spacing of $\Delta x = 0.65 \text{ mm}$, corresponding to 200 lattice points across the STR diameter (LX 200, approx. $11 \cdot 10^6$ lattice sites in the working volume) is employed. In contrast, Chapter 6 uses a mesh with 500 lattice points across the diameter (LX 500, approx. $265 \cdot 10^6$ lattice sites in the working volume),

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corresponding to $\Delta x = 4.00$ mm. In both studies, particle densities from experiments are largely matched in the simulations. Moreover, regardless of the particle type used, particle and fluid data is recorded only after a quasi-stationary state is reached in the mixed STR.

Fluid-particle interactions in numerical simulations

In order to understand the fluid-particle interaction and the eventual movement of an inertial or discrete particle in the surrounding fluid flow, Figure 2.10 describes the basic idea of the immersed boundary method (IBM) utilized herein.

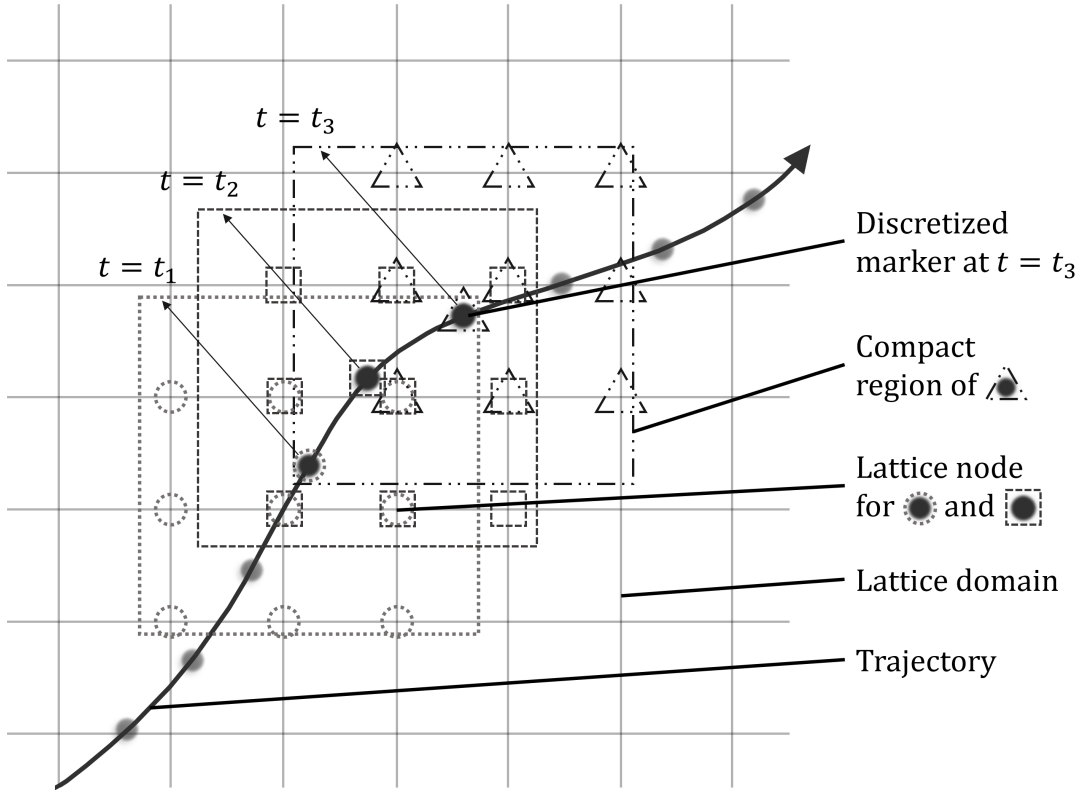


Figure 2.10: Two-dimensional illustration of a Lagrangian particle trajectory with discretized markers (filled circles) at three consecutive time steps $t \in \{t_1, t_2, t_3\}$ in the lattice domain. For each time step, a respective compact region (outer squares) with its surrounding lattice nodes in the D3Q19 structure (hollow symbols: circle, square, triangle) is depicted according to Favier et al. [Fav14].

The IBM was first presented by Peskin to model blood flow in a human heart [Pes72] and later was coupled with the LB method by Feng and Michaelides for particle-laden flows [Fen04, Fen05]. Basically, the IBM is employed to model moving surfaces with a deformable physical boundary that have high stiffness. Hence, particles are represented

Theoretical background

by Lagrangian points (discretized markers) that continuously flow through space over time (trajectory) within a fixed and uniform lattice domain. The flow field is only calculated at the lattice nodes, whereas Equation 2.15 and the no-slip approach is applied to the continuously moving discretized markers, which can have any location. Eventually, the boundary force density from the discretized marker is distributed to the neighboring lattice nodes within the compact region using the D3Q19 scheme. The velocity at the location of the discretized marker is then retrieved from the surrounding lattice nodes via an SGS model, allowing the fluid-particle interaction to be described and updated at each time increment [Guo13, Fav14]. Figure 2.10 illustrates this with an exemplary discretized marker on its trajectory shown at three consecutive time steps $t \in \{t_1, t_2, t_3\}$, together with the corresponding lattice nodes (circle, square, or triangle) defined by the shifting compact region. In the case of discrete element particles with $d_p > \Delta x$, the IBM treats the surface of the particle as a set of Lagrangian points (Fig. 2.9 d), thick circle) that are advected by the fluid instead of utilizing the Eulerian lattice nodes and a coarse computational boundary based on bounce-back rules of the LB method (Fig. 2.9 d), gray squares) [Fen05, Fen04].

In essence, in biotechnological processes, a detailed mechanistic understanding of mass and heat transfer in the culture broth is of importance, in particular on an industrial scale. Numerical simulations offer great possibilities but depend strongly on the quality of the underlying models [Box76] and on the data used for their calibration and validation. To date, however, no standard procedure has been specified [Kus21]. In 2016, The U.S. Food & Drug Administration (FDA) published a guidance on how to report computational modeling studies with respect to standardized reporting practices to ensure submissions include all the necessary details for reviewers to understand and evaluate a study [U.S16]. The most recent 2023 guidance shifts from simply reporting to a more comprehensive framework for evaluating credibility by emphasizing the importance of assessing whether the model used is reliable and suitable for its intended use, including model verification, validation, and uncertainty quantification [U.S23]. Hence, with respect to mixing time studies in STRs [Har25], incorporated particles of any size and density [Kus21], dense bubbly flows [Wei24], predicting hydrodynamic stress [Šro24], or predicting convective heat transfer coefficients [Tho24], validation studies are essential and constitute a central part of this work.

To ensure that experimental and simulated particle behavior and obtained hydrodynamic information are physically interpretable, it is essential to embed them within a consistent theoretical framework. This becomes especially important when evaluating results across multiple scales, from micro-scale particle motion to macro-scale hydrodynamics. Hence, fundamentals of the Eulerian and Lagrangian evaluation approach, relevant dimensionless

2.5 Governing evaluation frameworks, dimensionless numbers, and length scales for a multi-scale analysis

numbers, and characteristic length scales need to be introduced. These form the foundation for a structured multi-scale bioreactor analysis.

2.5 Governing evaluation frameworks, dimensionless numbers, and length scales for a multi-scale analysis

In order to interpret and analyze the resulting particle trajectories and flow structures in a physically meaningful way, it is essential to integrate the tracking data within an appropriate evaluation framework. Depending on whether the focus lies on fixed spatial positions or on individual particles moving with the flow, either the Eulerian or the Lagrangian evaluation is applied in this work, respectively. Moreover, various dimensionless numbers such as the Stokes number, Archimedes number, different Reynolds numbers, and associated characteristic length scales are introduced to conduct a multi-scale trajectory analysis.

The Eulerian and Lagrangian evaluation approach

The two primary methodologies for investigating fluid or particle flow are the Eulerian and Lagrangian approaches, as illustrated in Figure 2.11. In the Eulerian framework (Fig. 2.11 b)), the designated region of interest is subdivided into a predefined number of fixed control volumes (using a mesh or grid) through which the fluid moves. The observation of the temporal variation of the flow occurs from a stationary perspective while the fluid parcels pass by. Within each control volume, spatially averaged values of recorded target quantities, such as velocity, acceleration, dissolved oxygen, pH, or salinity, are calculated. In contrast, the Lagrangian framework (Fig. 2.11 c)) adopts the perspective of the fluid parcels themselves. Without relying on a predefined grid or control volumes, target quantities are recorded along the trajectories of individual fluid parcels as they move through the region of interest.

Passive tracers, such as very small, neutrally buoyant, and spherical particles, are commonly utilized in experimental techniques such as Particle Image Velocimetry (PIV) and Particle Tracking Velocimetry (PTV). In this ideal case, the tracer velocity matches the local fluid parcel velocity (Fig. 2.11 a)), and thus the tracers' trajectories directly represent the flow field. The tracer particles are therefore perfectly flow-following.

In order to understand the correlation between a spherical particle's flow-following behavior and its size and density, the Maxey-Riley equation (Equ. 2.14) can alternatively be stated as

$$(m_p + \frac{1}{2} m_f) \frac{d\mathbf{v}}{dt} = (m_p - m_f) \mathbf{g} + \frac{3}{2} m_f \frac{D\mathbf{u}}{dt} - 3\pi d_p \nu \rho_f (\mathbf{u} - \mathbf{v}), \quad (2.18)$$

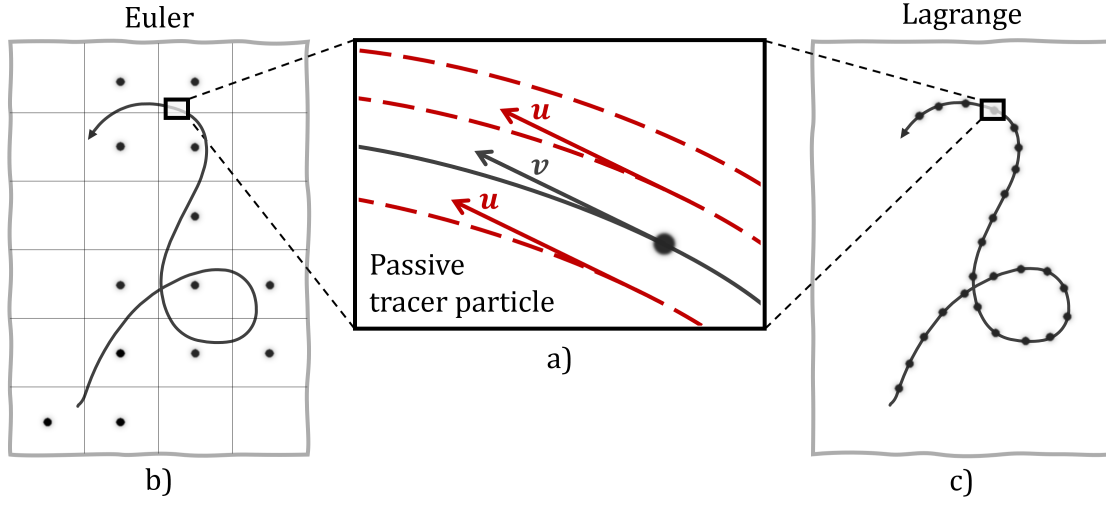


Figure 2.11: a) Velocity of the passive tracer particle $\mathbf{v}$ matches the velocity of the surrounding fluid parcel $\mathbf{u}$; b) Exemplary tracer particle trajectory in the Eulerian framework: the spatial average of recorded target quantity is depicted by the assigned dots in the center of each passed grid cell; c) Exemplary tracer particle trajectory in the Lagrangian framework: the target quantity is recorded along the particle's trajectory at each time step.

if a creeping flow is assumed ($Re_p < 1$, Equ. 2.25), and if all Faxén corrections and the time history (Basset) force are neglected. In this equation, the particle has a mass m_p , a velocity $\mathbf{v}$, and a diameter d_p , while the fluid has a mass m_f , a velocity $\mathbf{u}$, and a kinematic viscosity ν . With further introduction of the particle response time

$$\tau_p = \frac{d_p^2}{18\nu} \frac{\rho_p}{\rho_f}, \quad (2.19)$$

which is the time a particle needs to respond to the instantaneous change of velocity of the fluid [Cro11], and has its origin in the Maxey-Riley equation [Hof22b], Equation 2.18 can be rearranged so that the slip velocity $\mathbf{V}$ can be estimated as

$$\mathbf{V} = \mathbf{u} - \mathbf{v} = - \left(\frac{\rho_p - \rho_f}{\rho_p} \right) \tau_p \mathbf{g} - \frac{3}{2} \frac{\rho_f}{\rho_p} \tau_p \frac{D\mathbf{u}}{Dt} + \left(\frac{\rho_p + \frac{1}{2}\rho_f}{\rho_p} \right) \tau_p \frac{d\mathbf{v}}{dt}. \quad (2.20)$$

Assuming neutral buoyancy of the particle ($\rho_p \approx \rho_f$), the latter Equation 2.20 reduces to

$$\mathbf{V} = \frac{3}{2} \tau_p \left(\frac{d\mathbf{v}}{dt} - \frac{D\mathbf{u}}{Dt} \right). \quad (2.21)$$

2.5 Governing evaluation frameworks, dimensionless numbers, and length scales for a multi-scale analysis

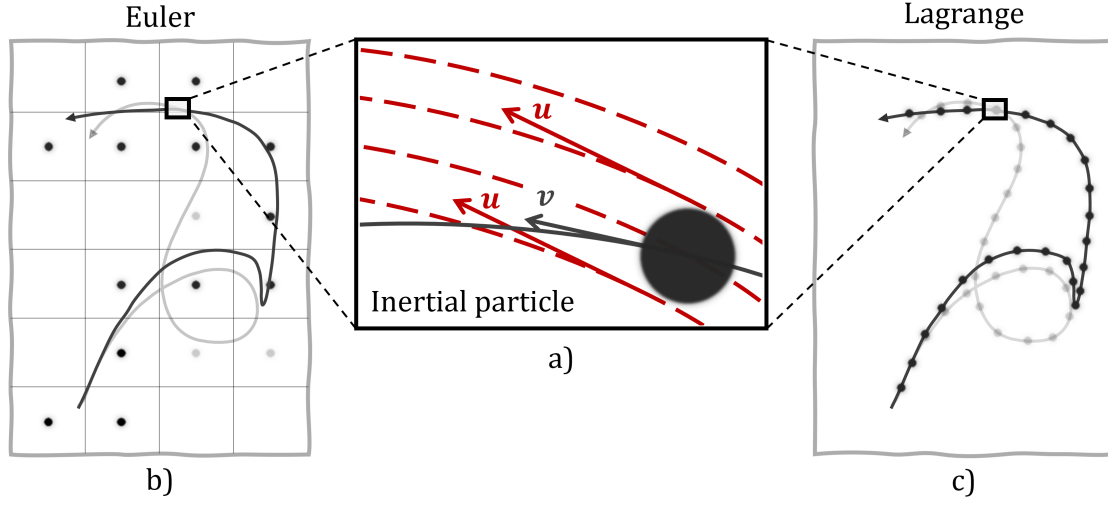


Figure 2.12: a) Velocity discrepancy between the inertial particle's velocity $\mathbf{v}$ and the surrounding fluid velocity $\mathbf{u}$, adapted from Tropea et al. [Tro07]. b) Exemplary inertial particle trajectory in the Eulerian framework: the spatial average of the recorded target quantity is depicted by the assigned dots at the center of each passed grid cell; transparent line and dots indicate the trajectory and recorded target quantity of the passive tracer particle, respectively; c) Exemplary inertial particle trajectory in the Lagrangian framework; transparent line and dots indicate the trajectory and recorded target quantity of a passive tracer particle, respectively.

As mentioned in Section 2.4, the Maxey-Riley equation is valid only for very small particles. Nevertheless, considering those assumptions, in the latter expression it is evident that the particle diameter d_p in the particle relaxation time τ_p must be sufficiently small to minimize the slip velocity V and thereby ensure ideal flow-following behavior. Finite particle size and density deviations, however, introduce inertial effects that cause discrepancies between particle and fluid velocities. These arise because the stresses imposed by the fluid flow are effectively averaged across the surface area of the particle [Oue08, Qur07]. Consequently, when particles are larger or when the density ratio $\rho_p \rho_f^{-1}$ exceeds unity, their trajectories deviate from the instantaneous streamlines of the fluid (Fig. 2.12 a)). Relative to the surrounding carrier fluid, they exhibit inertial behavior and respond with a delay to changes in the underlying flow field as their particle response time τ_p increases.

Figures 2.12 b) and c) demonstrate this effect figuratively from a Eulerian and a Lagrangian point of view, respectively. This impact can be experimentally investigated by demonstrating a reduction in the variance in particle acceleration [LP01, Qur07, Oue08, Cal09] (Chap. 4) or clustering behavior within specific regions of the flow (Chap. 6) [Bec05, Bec14, Pet19].

Theoretical background

Depending on the size and density of the particle and the Reynolds number, the effectiveness of the particle to follow the flow is determined by the dimensionless Stokes number St . It represents the ratio of the particle response time τ_p to the characteristic time of the fluid flow τ_f as

$$St = \frac{\tau_p}{\tau_f}. \quad (2.22)$$

Hence, a low Stokes number ($St \ll 1$) indicates a strong coupling to the motion of the fluid (Fig. 2.11), while a high Stokes number ($St \gg 1$) implies that particle inertia dominates and the particle decouples from local velocity fluctuations (Fig. 2.12).

In addition, the influence of the density difference is captured by another dimensionless number, the Archimedes number Ar , which is defined as

$$Ar = \frac{\rho_f (\rho_p - \rho_f) g d_p^3}{\eta^2}, \quad (2.23)$$

and describes the ratio of gravitational (buoyancy-driven) forces to viscous (frictional) forces [Kal20]. It is commonly used to characterize particle settling phenomena and also as a criterion for particle off-bottom lifting in stirred tank reactors (STR). Mersmann et al. proposed a theoretical prediction based on this dimensionless number to estimate the minimum specific power input required to fully suspend the particles, as well as the corresponding particle volume fraction [Mer98].

Reynolds numbers and drag coefficient

In order to characterize a moving flow, the Reynolds number Re is used to determine whether a flow is laminar, transitional, or turbulent. This dimensionless number is the ratio between the prevailing inertial force and the viscous force in a finite fluid element. Since in this thesis different STRs are characterized and discussed, the stirrer Reynolds number

$$Re_{STR} = \frac{\rho_f n d_{imp}^2}{\eta} \quad (2.24)$$

describes the flow regime in a bioreactor as a whole, in which an agitator with one impeller or a multistage impeller configuration stirs the fluid with the impeller frequency n and the impeller diameter d_{imp} . Regarding the flow classification, the turbulent region is reached for a specific impeller configuration in a defined fluid, once the impeller frequency is independent of the power input P into the stirred system. In a single-phase, baffled STR filled with a Newtonian fluid, this is mostly at $Re_{STR} \approx 10^4$ [Zlo01, Eka12].

2.5 Governing evaluation frameworks, dimensionless numbers, and length scales for a multi-scale analysis

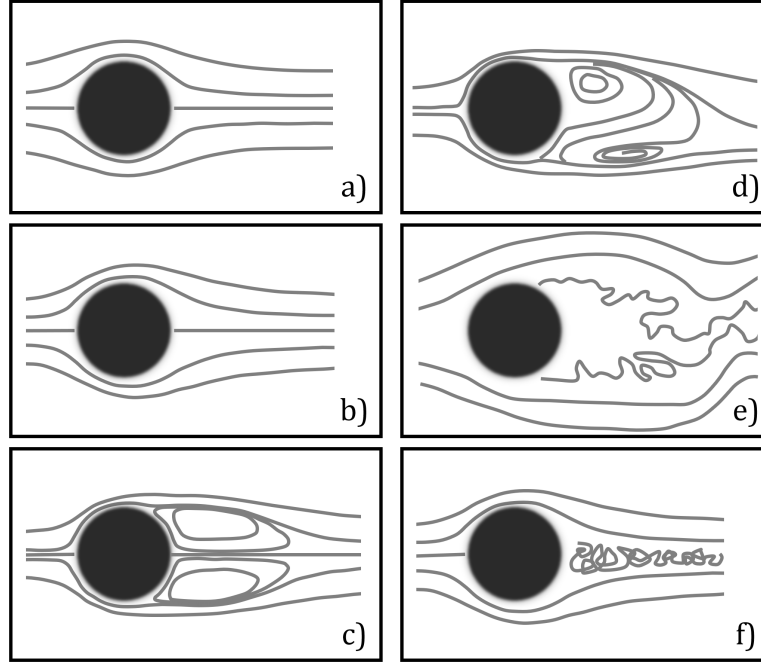


Figure 2.13: Exemplary depiction of the flow regimes around a sphere: a) $Re_p < 1$, Stokes flow; b) $1 < Re_p < 20$, unseparated flow; c) $20 < Re_p < 130$, steady wake region; d) $130 < Re_p < 400$, onset of wake instability; e) $400 < Re_p < 3.5 \cdot 10^5$, high subcritical Reynolds number range; f) $Re_p > 3.5 \cdot 10^5$, critical transition and supercritical flow [Cli78, Hou03, Ric12].

To be able to characterize the flow around incorporated particles, the particle Reynolds number

$$Re_p = \frac{\rho_f d_p V}{\eta} \quad (2.25)$$

constitutes the dynamics of the actual particle in the surrounding fluid flow with the slip velocity V . As exemplarily depicted in Figure 2.13, Clift et al. describe six regimes around a spherical particle, namely the Stokes flow regime ($Re_p < 1$, Fig. 2.13 a)), the unseparated flow ($1 < Re_p < 20$, Fig. 2.13 b)), the steady wake region ($20 < Re_p < 130$, Fig. 2.13 c)), the onset of wake instability ($130 < Re_p < 400$, Fig. 2.13 d)), the high subcritical Reynolds number range ($400 < Re_p < 3.5 \cdot 10^5$, Fig. 2.13 e)), and the critical transition and supercritical flow ($Re_p > 3.5 \cdot 10^5$, Fig. 2.13 f)) [Cli78].

Most importantly, for intermediate and high Reynolds numbers ($1 < Re_p < 3.5 \cdot 10^5$), for which Stokes drag no longer applies, the drag force F_D being part of the quasi-steady viscous force term (Equ. 2.14), requires corrections for non-linear effects accounting for the

Theoretical background

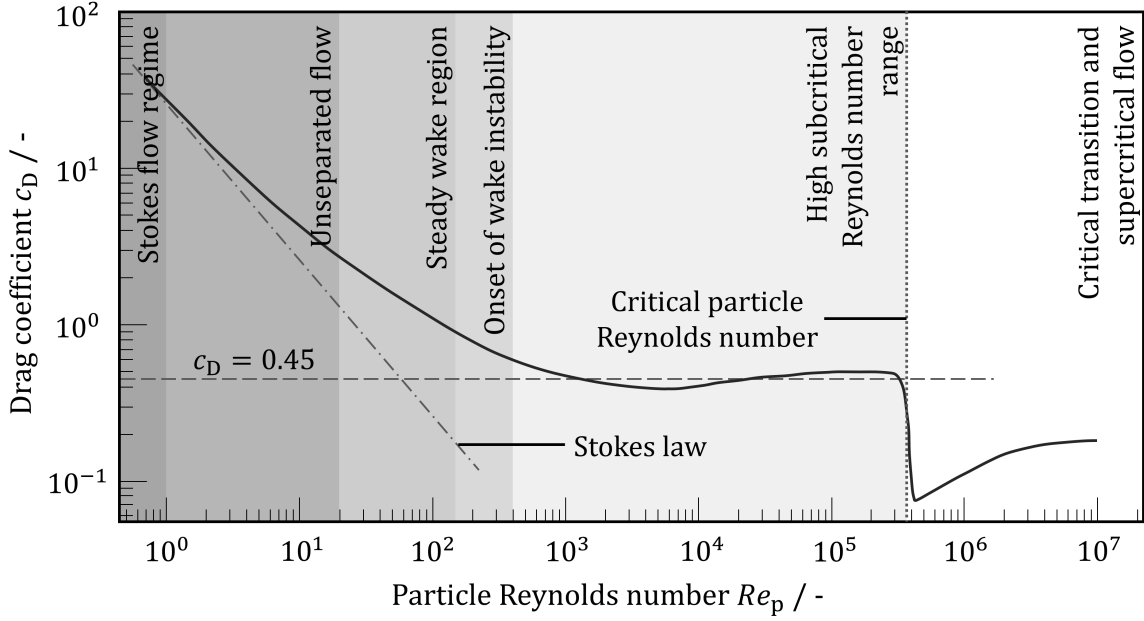


Figure 2.14: Exemplary depiction of the drag coefficient c_D as a function of the particle Reynolds number Re_p for a non-rotating, spherical particle, according to Crowe et al. [Cro11].

velocity-dependent drag as

$$\mathbf{F}_D = \frac{\pi}{8} c_D d_p^2 \rho_f |\mathbf{u} - \mathbf{v}| (\mathbf{u} - \mathbf{v}). \quad (2.26)$$

The drag coefficient c_D is a function of the particle Reynolds number Re_p and therefore strongly depends on the flow around the particle, but also on its shape and sphericity ϕ .

As seen in Figure 2.14, the linear correlation $c_D = 24 Re_p^{-1}$ applies to the Stokes flow regime (creeping flow) [Bra72]. As the Reynolds number increases, the drag coefficient tends to approach a nearly constant value until a sudden drop occurs at the critical particle Reynolds number. Multiple empirical correlations are found in the literature for modeling the drag coefficient c_D , such as

$$c_D = \frac{24}{Re_p} \left(1 + 0.15 Re_p^{0.687} \right), \quad (2.27)$$

which has less than 5 % range of deviation for $1 < Re_p < 800$ [Sch33], or

$$c_D = \frac{24}{Re_p} \left(1 + a \cdot Re_p^b \right) + \frac{c}{1 + \frac{d}{Re_p}}, \quad (2.28)$$

2.5 Governing evaluation frameworks, dimensionless numbers, and length scales for a multi-scale analysis

which is a four-parameter (a, b, c, d) general drag correlation, for which values according to Haider and Levenspiel [Hai89] or Turton and Levenspiel [Tur86] can be utilized with less than 3 % range of deviation for $Re_p < 2.6 \cdot 10^5$ [Cli78].

Characteristic length scales in stirred tank reactors

In order to apply a multi-scale analysis approach, different characteristic length scales need to be introduced as they characterize the investigated system and are eventually utilized in case of a scale-up or scale-down in the Stokes number, and the particle Reynolds number.

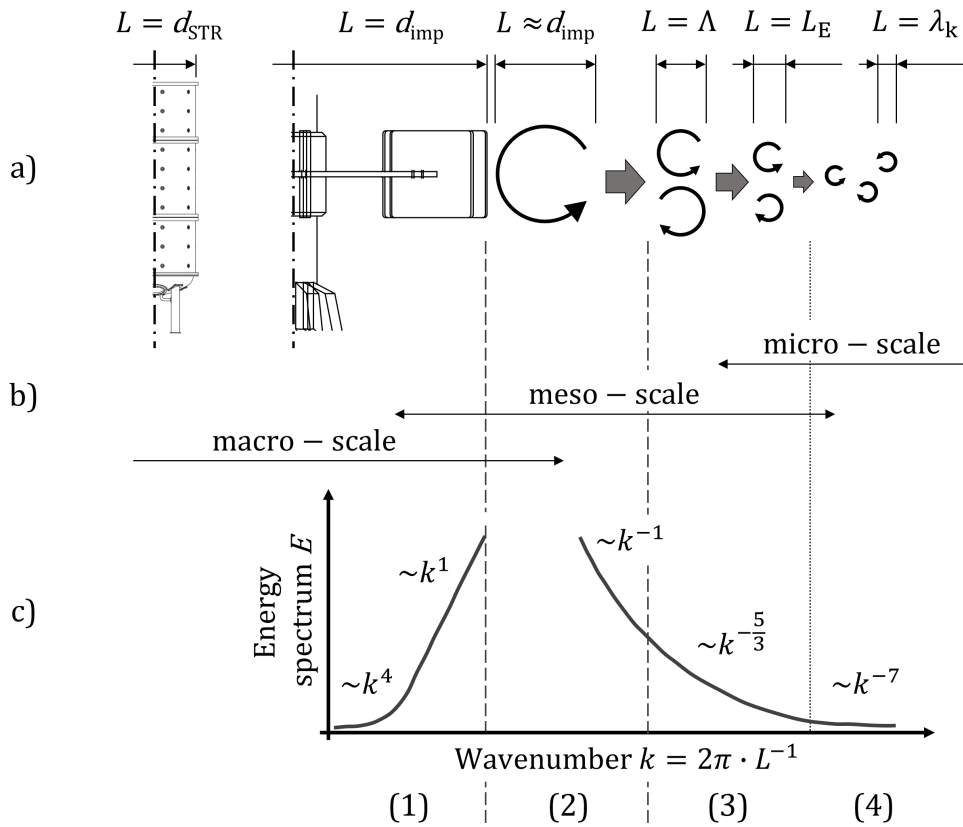


Figure 2.15: a) Exemplary depiction of decreasing length scales L occurring in a bioreactor and the vortex decay via an energy cascade [Ric07]: the largest length scale is the reactor diameter d_{STR} , continuing to the energy injection by the impeller with its diameter d_{imp} and the respective vortex size, further decreasing to the integral length scale Λ , and the Taylor length scale L_E toward the smallest vortex at the Kolmogorov length scale λ_k ; b) Classification of the length scales into three coarse ranges used in this thesis, such as macro-scale, meso-scale, and micro-scale; c) Theoretical energy spectrum range E as a function of the wavenumber k divided into four ranges: (1) large eddies range, (2) energy-containing eddies range, (3) inertial subrange, and (4) viscous dissipation subrange, where the last two can be summarized into the universal equilibrium range [Kim64, Kra02, Boc10, Wol10, Rei13].

Theoretical background

Figure 2.15 a) illustrates exemplary the different length scales L that occur in turbulent mixing processes and are utilized in calculations, in particular for the estimation of the dimensionless Stokes number St . The boundary conditions in an STR are, on the one hand, the largest eddies at the size of the impeller, and, on the other hand, the smallest eddies at the size of the Kolmogorov length scale λ_k . Therefore, a coarse distinction is made between the macro-, the meso- and the micro-scale (Fig. 2.15 b)). At the impeller blade, which has a diameter of d_{imp} , anisotropic, energy-rich, and large vortices are generated at the same order of magnitude as the impeller [Zlo01]. According to the vortex decay hypothesis, those generated vortices within the system are inherently unstable and break down to smaller vortices, cascading the kinetic energy of the flow to progressively smaller scales [Ric07], as depicted theoretically in Figure 2.15 c). At the largest scales of the system, in the large eddies range (1), the kinetic energy is added to the system, where these scales reach a statistical equilibrium [Ale18]. In the energy-containing eddies range (2), the forcing injection takes place and the energy is highest. Continuing to decrease the scale, the universal equilibrium range stretches approximately from the integral length scale $\Lambda \approx 0.1 \cdot d_{\text{imp}}$ [Och08], and the Taylor length scale L_E at the inertial subrange (3), further down to the viscous dissipation subrange (4) [Kim64]. In the latter, the kinetic energy dissipates into thermal energy due to viscous forces, which assumes local isotropic turbulence [Kol58]. Hence, the energy distribution is a function of the vortex size, and thus the wavenumber k .

Herein, the Taylor length scale [Tay35] characterizes the length at which viscous dissipation begins to influence turbulent eddies [Hui21] and is estimated as

$$L_E = \mathbf{u}'_{\text{rms}} \cdot \sqrt{\frac{15 \nu}{\bar{\varepsilon}_T}}, \quad (2.29)$$

where $\mathbf{u}'_{\text{rms}}$ denotes the root mean square of the fluid velocity fluctuations, ν is the kinematic viscosity, and $\bar{\varepsilon}_T$ is the mean energy dissipation rate, given by $\bar{\varepsilon}_T = P \cdot (V \rho_f)^{-1}$ with P being the global power input, V the reactor volume, and ρ_f the fluid density.

Building on the physical analysis of particle-laden flows, the computation of temporally resolved particle locations, and subsequent evaluations in Eulerian and Lagrangian frameworks across scales, these findings can now be set in a broader context. The insights obtained provide the necessary foundation for understanding global and local flow structures. By incorporating laboratory- and industrial-scale particle trajectory data as well as local mixing information, it becomes possible to assess particle exposure times to critical process regimes. Such trajectory-based analyses offer a valuable opportunity to integrate knowledge of mixing performance, identified heterogeneities in form of compartments, and Lagrangian particle

information to eventually derive a deeper understanding of performance losses during the scale-up of biopharmaceutical production processes.

2.6 Trajectories and lifeline analysis

The study of trajectories has a long history, originating in geophysical contexts such as the measurement of ocean currents using drifters. In these applications, they follow the flow of water in their characteristic manner and record data about their location, subsurface temperature, salinity, dissolved carbon dioxide, and many more (Fig. 2.16 a) and d)) [Lum07, Bou08]. Those experimental measurements, in addition to satellite observation data, support Lagrangian models to investigate large-scale transport processes and eventually describe transport and mixing phenomena in the field of oceanography [Huh12, Hal15].

In process engineering, and particularly in biotechnological applications where experimental data on cell behavior and residence times in critical regimes are lacking, Lagrangian data provide unique insights into flow structures by capturing the journey of particles within the carrier flow. In the publications presented here, different types of particles are used to capture Lagrangian data at both laboratory and industrial scale, as shown in Figure 2.16 b) and c), respectively. On laboratory scale, fluorescent particles such as calcium alginate beads are used in a 4D-Particle Tracking Velocimetry (4D-PTV) setup (Chap. 4). On industrial scale, encapsulated, instrumented particles such as Lagrangian Sensor Particles (LSP) are used (Chap. 5 and 6). Figure 2.16 c) shows a prototype LSP equipped with an Adafruit MPRLS pressure sensor, a BlueDot BNO055 Inertial Measurement Unit, a SparkFun Openlog data logger, an ATMEL ATtiny85 microcontroller, a 290 mAh Lithium Ion Polymer battery, and two red LEDs. However, this prototype is not used in the present work. Consequent lifelines are illustrated in Figure 2.16 e) and f). The first depicts lifelines obtained using the 4D-PTV measurement technique, while the second shows a long-term exposure image of LSPs traversing through an acrylic glass stirred tank reactor (STR), made visible by their respective LED color.

Blöbaum et al. published a review paper on microbial lifelines, in which lifeline determination concepts and lifeline analysis applications are outlined [Blö23]. In the following, the origin and definition of lifelines and the different lifeline determination measures are summarized. For more details, the reader is referred to the original review manuscript.

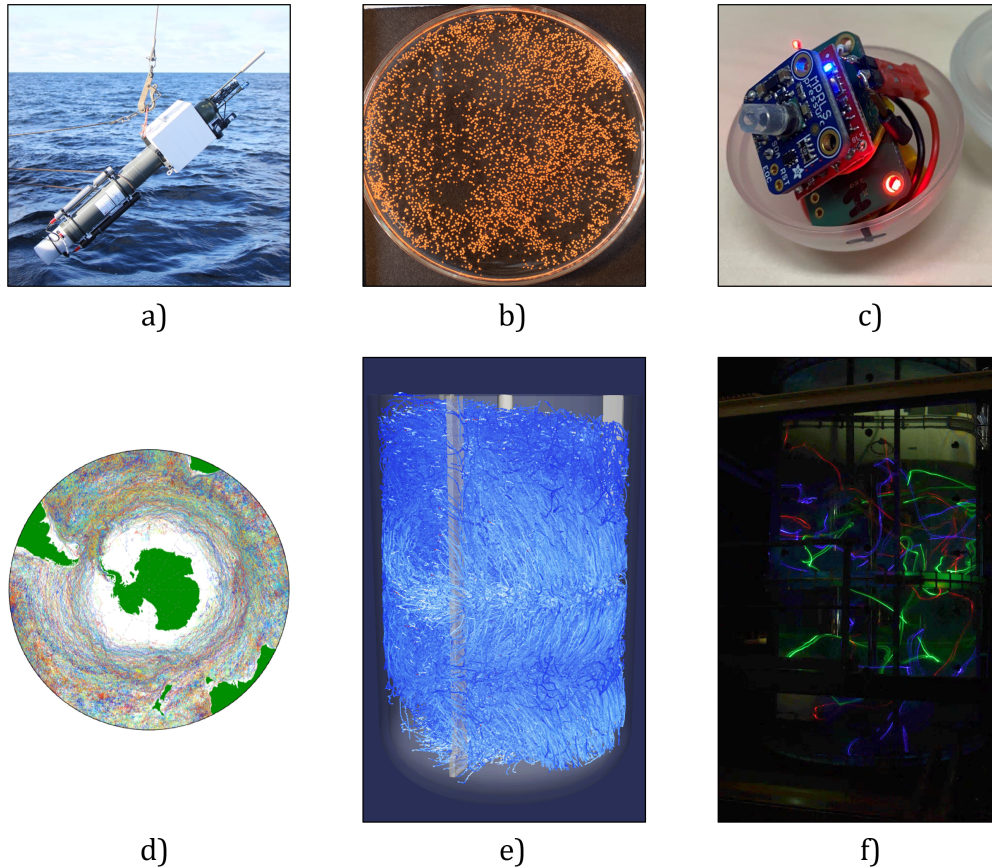


Figure 2.16: a) Biogeochemical Argo float deployment to collect data in the ocean (Photo: Leibniz Institute for Baltic Sea Research Warnemünde, M. Naumann) [Lei22]; b) Orange-fluorescing calcium alginate beads for 4D-Particle Tracking Velocimetry; c) Opened LED Lagrangian Sensor Particle prototype equipped with various electronic components including two red LEDs; d) Map of all Southern Ocean observational Lagrangian surface drifters in the National Oceanic and Atmospheric Administration Global Drifter Program (NOAA GDP) dataset [Lum07, VS18]; e) Over 20,000 cumulated experimentally obtained Lagrangian trajectories in a 3 L bioreactor using 4D-Particle Tracking Velocimetry; f) Long-term exposure image of the 15,000 L acrylic glass bioreactor with three deployed LED LSPs, each with two red, green, or blue LEDs, respectively.

Utilization of lifeline analysis in biotechnology

In biotechnological process engineering, particularly in the operation of STRs, Lapin et al. published an article in 2004, in which they referred to the journey of massless tracers, or rather individual *Saccharomyces cerevisiae* cells and their environmental condition in a bioreactor to "lifelines". They applied a Euler-Lagrange approach to investigate population behavior based on single-cell trajectories in space and time. This approach allowed for an explanation of the cell-bioreactor interaction, describing the relationship between the

2.6 Trajectories and lifeline analysis

intracellular state and the surrounding turbulent flow field [Lap04]. Since then, the word "lifeline" has been utilized in numerous publications. However, its definition differs and is ascribed to different meanings. According to Blöbaum et al., the authors utilize "lifeline" either for

- i) describing trajectories through environmental gradients,
- ii) demonstrating the cell's perception of its time-dependent environmental condition,
- iii) evaluating the cell's metabolic condition in time, or
- iv) a mixture between those [Blö23].

Notably, environmental and intracellular lifelines are closely interconnected, as environmental gradients shape intracellular responses and vice versa. This work focuses primarily on the first type: lifelines as trajectories of differently sized particles (Fig. 2.16 b) and c)) that are utilized to mimic cells in the reaction volume. Additionally, consequential temporal environmental conditions along the trajectory lead to estimations of the cell's perception to measured gradients, and thus broaden the hydrodynamic understanding of the bioreactor-cell interaction on different scales.

Haringa et al., predominantly focused on developing numerical models for industrial fermentation processes, and advanced lifeline analyses by introducing three statistical evaluation methods. These methods are designed to analyze lifelines and the accompanying fluctuations in the external environment that cells experience as they traverse the bioreactor during cultivation [Har18a] and are called

- i) "regime analysis", which involves discretizing lifelines based on their residence time τ in the respective metabolic regime "excess" ($\mu > 0.95 q_{s,\max}$), "limitation" ($0.95 q_{s,\max} > \mu > 0.05 q_{s,\max}$), and "starvation" ($\mu < 0.05 q_{s,\max}$), where μ is the specific cell growth rate and $q_{s,\max}$ is the maximum specific uptake rate of a substrate [Har17].
- ii) "arc analysis", which provides a distribution of fluctuation durations specifically within the "limitation" regime, based on a defined baseline ratio between the specific substrate uptake rate q_s and $q_{s,\max}$ (e.g. $q_s q_{s,\max}^{-1} = 0.5$) [Har16], and
- iii) "Fourier analysis", which is mainly suited for highly periodic behaviors and narrow circulation time distribution (CTD), such as occurring in airlift reactors [Har16].

In this work, the "regime analysis" approach is demonstrated to be a valuable method. However, rather than evaluating metabolic regimes based on cellular metabolic responses,

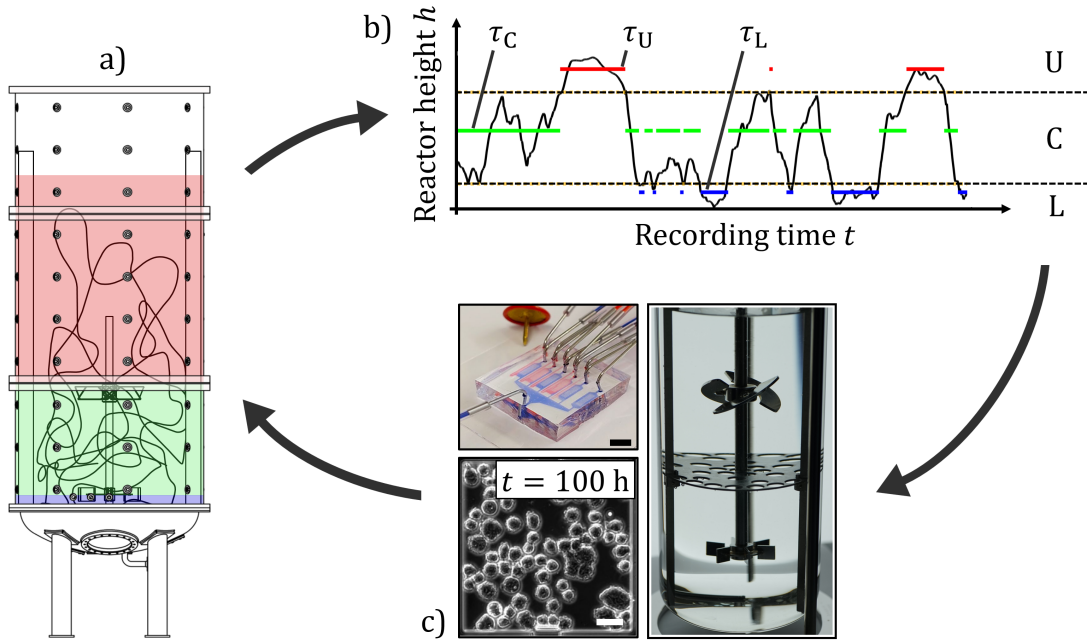


Figure 2.17: a) Exemplary depiction of a cell lifeline passing through three determined compartments in the STR (upper: red, center: green, lower: blue); b) Translated LSP lifeline over the reactor height as a function of the recording time with discretized regime residence times τ in the respective upper compartment (U, red), center compartment (C, green), and lower compartment (L, blue); c) Gradients observed at large scale are translated into laboratory-scale investigations using, for example, microfluidic cultivation devices (top left, black bar: 5 mm) [Blö24], where Chinese hamster ovary cells can grow in microcolonies under controlled environmental conditions (bottom left, white bar: 30 μm) [Sch24], or scale-down reactors (right) [Gau22], in which large-scale hydrodynamics can be mimicked.

hydrodynamic regimes and their boundaries are assessed using LSP circulation time distribution (CTD) data, LSP residence time distribution (RTD) data, and, where applicable, local mixing time distributions for the validation of respective boundaries. The resulting vertical compartment boundaries separate prevailing hydrodynamic regions and reveal potential excess or starvation regimes, such as theoretical oxygen deprivation zones.

As exemplary depicted in Figure 2.17 a), the identified compartments are treated as equivalents to regimes, since transport barriers are observed between them, indicating that substrate transport is likely restricted. To this end, discretizing Lagrangian LSP lifeline data into an upper (U, red), center (C, green), and lower (L, blue) regime (Fig. 2.17 b)) yields regime residence times for the respective compartment $\tau \in \{\tau_U, \tau_C, \tau_L\}$. This distribution from the regime analysis provides valuable information about temporal fluctuations between specific environmental conditions. Hence, not only the axial position of a transport barrier can be derived, but also a statistical analysis of cells encountering particular substrate

conditions, as similarly described by Haringa et al. and demonstrated in Chapters 5 and 6. Eventually, these data can be utilized for the development and application of microfluidic devices (Fig. 2.17 c) left), which in turn mimic measured fluctuating environments in a controlled way and apply it to living microorganisms (e.g. Chinese hamster ovary cells) to study intracellular dynamics [Ho22, Blö24, Sch24, God09b]. Another way of utilizing the knowledge about a regime analysis is through scale-down bioreactors (Fig. 2.17 c) right). These customized reactor types mimic large-scale gradients on laboratory scale by means of compartment discs and offer great opportunities to optimize the original process, additionally adapt and optimize cell strains, and even develop new bioprocesses for a characterized reactor.

Translating large-scale heterogeneities into scale-down experiments

From a Lagrangian perspective, as cells traverse throughout a bioreactor, they undergo transient oscillations in environmental conditions and are therefore exposed to suboptimal conditions, which are recognized for their adverse effects [Ana21, Gao16, Lar06]. Especially in large-scale bioreactors, mixing often requires tens to hundreds of seconds, whereas key intracellular processes occur within only a few seconds, which promotes population heterogeneity [Hei18]. In order to tackle this problem efficiently, the approach is to replicate large-scale conditions on laboratory scale as this environment allows for easier control, adjustment, and precise measurement. Those scale-down experiments, associated reactor designs, and methodologies are described in detail by Nadal-Rey et al. [Nad21] and Neubauer and Junne [Neu16].

The most common approach of mimicking large-scale conditions is utilizing multicompartiment systems, which comprise multiple sequential STRs or a combination of STRs and plug-flow reactors interconnected by pump lines. They allow investigating the impact of hypoxia, pH fluctuations, or other stress conditions on microorganisms and additionally offer the potential to select cells that demonstrate the highest resilience to fluctuating extracellular environments [Pau20]. However, in their studies on Chinese hamster ovary (CHO) cells and their physiological response to hydrodynamic stress, Godoy-Silva et al. reveal that exposure to intense energy dissipation rates in an external recirculation loop causes a shift in the glycosylation pattern, even though they use two orders of magnitude lower dissipation rates than those at which physical damage is observed [God09b, God09a]. Rather contrasting results are shown in a scale-down experiment using a peristaltic pump by Nienow et al.; however, sublethal damage to CHO cells is concluded when the reactor content passes through the pump, which in turn may mask the adverse effects of large-scale heterogeneities [Nie13]. Due to these overall deteriorating effects on the cell's integrity, the project partner in the

Theoretical background

subproject "CHOLife", Gaugler et al., investigate another type of scale-down simulator: the single multicompartment bioreactor (SMCB, Fig. 2.18) [Gau22, Gau24].

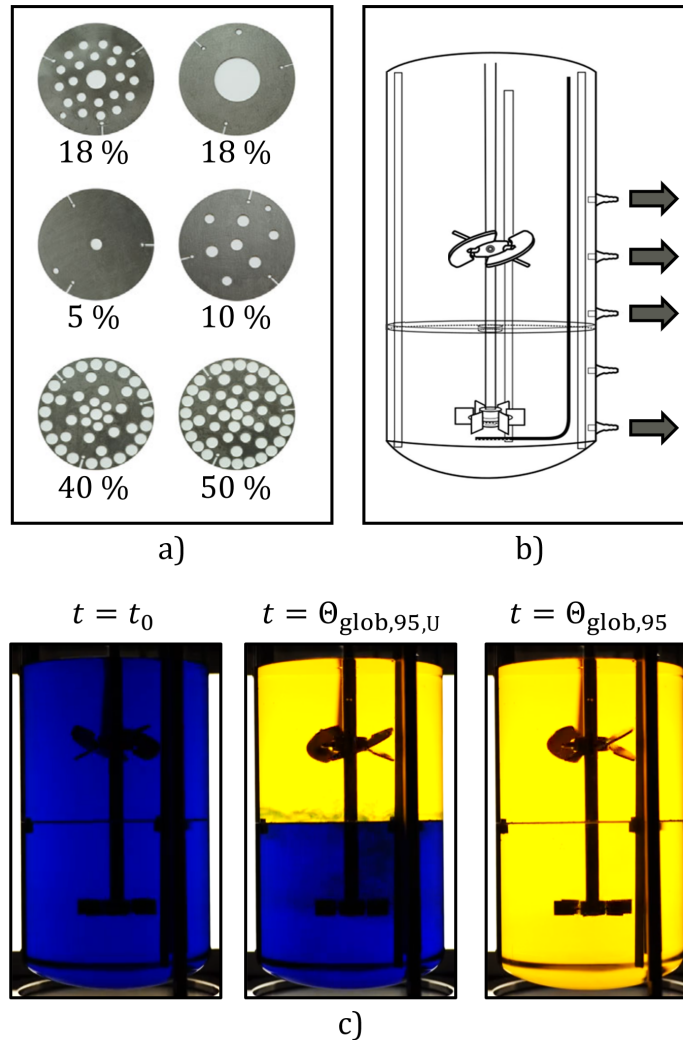


Figure 2.18: a) Stainless steel compartment discs with different exchange areas A_{ex} (indicated with percentages) to divide the bioreactor vertically and to obtain a determined exchange volume flow between the forced compartments; b) Schematic illustration of the single multicompartment bioreactor (SMCB) with an indicated compartment disc between both impellers and four outlet valves for sampling; c) Exemplary depiction of a mixing time trial with bromothymol blue at three consecutive time steps at the starting point t_0 , global mixing time of the upper compartment $\Theta_{glob,95,U}$, and the global mixing time of the entire reactor volume $\Theta_{glob,95}$. All images are taken and modified from Gaugler et al. [Gau22, Gau24].

Following the design by Schilling et al. [Sch99], vertically stacked discs are integrated into the reactor to directly mimicking large-scale compartments within a single laboratory-scale bioreactor, without requiring physically separated STRs or external equipment such

as pumps. Figure 2.18 a) shows different types of stainless steel separation discs with the respective percentage of the total area allowing for a vertical volume flow exchange between the forced compartments. Figure 2.18 b) illustrates the customized SMCB with a disc between the Rushton turbine (lower impeller) and the segment stirrer (upper impeller). The black arrows indicate sample valves for a determination of the viable cell concentration (VCC), and a consecutive analysis of glucose, lactose, antibody (for glycan profiles), and fibronectin concentrations. The publication of Gaugler et al. describe how to replicate the industrial scale volumetric power input in the SMCB to enable comparable mixing times, as seen in Figure 2.18 c). Multiple disc designs with each having a characteristic exchange area A_{ex} , and thus a particular volume flow exchange, are scrutinized. Eventually, a decision tree is obtained for the customized selection of discs depending on large-scale measurements [Gau22]. Afterwards, CHO fed-batch cultivations are performed in the SMCB with two different disc designs (5 % and 10 % exchange area) as well as different volumetric power inputs (up to $P V^{-1} = 20.4 \text{ W m}^{-3}$) and compared to a reference bioreactor. The outcome is an 87 % rise in lactate accumulation, an elevated glucose uptake, along with a diminished VCC, which are all effects associated with large-scale cultivations. The glycosylation patterns analyzed reveal a changing degree of galactosylation rather depending on volumetric power inputs than on prevalent heterogeneities [Gau24]. To conduct further follow-up studies in the SMCB and to extend the current compartment model, the acquisition of more extensive large-scale data is essential.

To enable a representative scale-down approach, valuable large-scale information, such as axial flow velocities, mixing times per compartment, and the location and number of transport barriers are crucial. By utilizing the access to a 15,000 L industrial-scale bioreactor, Lagrangian Sensor Particles yield first-of-its-kind lifeline data that permit a direct hydrodynamic comparison of measurements across scales. The SMCB bridges the gap between lab-scale and production-scale CHO cultivations, offering a platform not only for mimicking complex environmental heterogeneities, but also for identifying cellular response patterns. By integrating metabolomics, proteomics, cell cycle studies, and transcriptional profiling, future SMCB cultivations will enable robust predictions of CHO performance.

Chapter 3

Thesis outline

In this thesis, the multi-scale experimental and numerical analysis of lifelines for a bioreactor characterization is demonstrated by three peer-reviewed publications in the following Chapters 4, 5, and 6. Each chapter corresponds to a research topic covering specific investigation frameworks (laboratory scale, industrial scale, Lattice-Boltzmann simulation) offering a thorough explanation of the motivation, a corresponding theoretical background, applied materials and methods, obtained results, their discussion and literature comparison, and conclusions. The chapters are interconnected thematically, each building upon the insights of the previous. The following three fundamental research questions (RQ) guide the focus of the studies with the ultimate goal of obtaining a fingerprint of a bioreactor by merging experimental and numerical data and to close the knowledge gap between the laboratory scale and industrial scale in terms of Chinese hamster ovary (CHO) cell metabolism when exposed to heterogeneities:

- RQ1: To what extent can state-of-the-art Lagrangian Sensor Particles (LSP) represent cell lifelines, and how does their inertial behavior influence this capability?
- RQ2: How reliably can accompanying Lattice-Boltzmann large eddy simulations capture the dynamics of inertial particles in bioreactors across scales, and what are their limits?
- RQ3: What characteristic flow structures and heterogeneities can be identified through Lagrangian lifeline analysis, and how can these insights support the understanding of industrial-scale bioreactors?

In order to classify the RQs stated and gain an overview, Figure 3.1 illustrates the interconnection between the three investigation frameworks presented in this thesis, which are the laboratory scale (pink), the industrial scale (blue), and associated Lattice-Boltzmann simulations (gray).

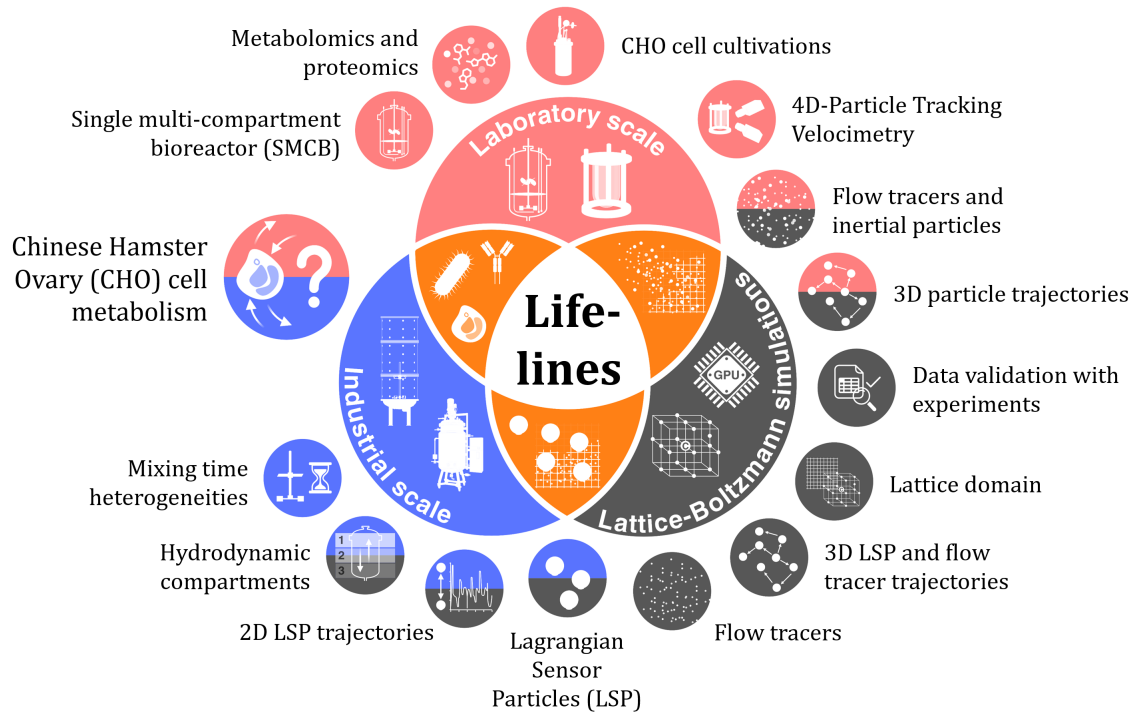


Figure 3.1: Figurative representation of the three investigation frameworks of this thesis. For the investigation of lifelines, experiments are performed on laboratory scale (pink) and on industrial scale (blue) with accompanying Lattice-Boltzmann simulations (gray). Three overlapping inner circles (orange) depict the bilateral material used. Outer circles illustrate materials, methods and outcomes of the respectively colored investigation framework [Hof25b].

All frameworks have in common that particle trajectories are measured, which essentially represent lifelines of cells in a characteristic manner. The overlapping inner areas (orange) depict the utilized material, which the respective frameworks have in common, and are either cells or differently sized particles. The top left overlapping material is that microorganisms produce monoclonal antibodies during a cultivation on laboratory scale and also in industrial-sized bioreactors. The second overlapping matter, top right, shows that flow tracers and inertial particles are applied in a laboratory-scale bioreactor. This experimental work is accompanied by Lattice-Boltzmann large eddy simulations (LB LES) using a lattice structure (indicated as a mesh grid) with a specific number of lattice points. The third overlapping material at the bottom illustrates, on the one hand, LSPs, which are applied on industrial scale as well as simulated by LB LES using a lattice structure, and, on the other hand, flow tracers, which are only introduced in LB LES. The outer circles denote the utilized materials, methods, and outcomes of the respectively colored investigation framework. The results obtained and discussed in this work eventually complement wet-lab studies conducted by the "CHOLife"

project partner Gaugler et al., who focus on developing a single multi-compartment bioreactor (SMCB) for scale-down experiments, including CHO cell cultivations followed by proteomic and metabolomic analyses to study cellular production.

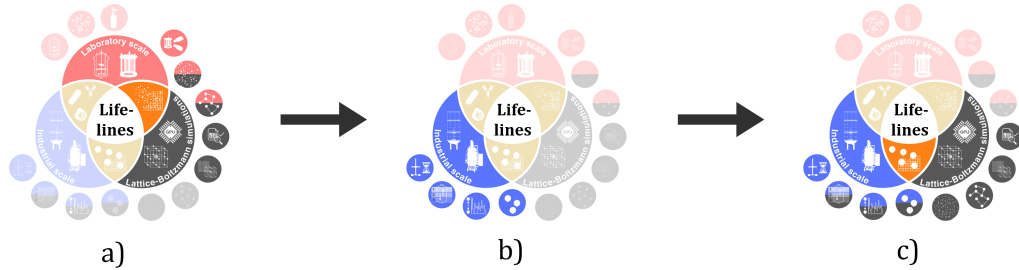


Figure 3.2: a) Covered investigation frameworks in following chapters: a) First publication: laboratory scale and accompanying Lattice-Boltzmann simulations, b) second publication: industrial scale investigations with Lagrangian Sensor Particles, c) third publication: industrial scale and accompanying Lattice-Boltzmann simulations [Hof25b].

The first publication, presented in Chapter 4, investigates particle trajectories on laboratory scale in a 3 L stirred tank reactor (STR), complemented by LB LES (Fig. 3.2 a)). It explores to what extent inertial particles follow the flow in the bioreactor used, and how their spatially evaluated Stokes number can be interpreted in the context of cell lifelines. For this purpose, small polyethylene particles and calcium alginate beads, which are approximately four times larger, are used, the latter representing laboratory-scale representatives of LSPs. Additionally, it is assessed how accurately LB LES reproduce experimental particle trajectories and what limitations emerge related to particle size within the discrete lattice domain. To bridge toward industrial scale, a theoretical maximum particle diameter is estimated, above which LSPs no longer fulfill flow-following behavior.

Chapter 5 presents the second publication, which reports on the first experimental LSP trials conducted in a 15,000 L STR (Fig. 3.2 b)). It addresses the question of how well LSPs follow the flow structures in the industrial-scale bioreactor and evaluates their suitability for representing cell lifelines under turbulent mixing conditions. Moreover, it critically examines the availability and interpretation of circulation time distributions and, for the first time, applies an experimental Lagrangian regime analysis to obtain valuable information for scale-down reactor designs, with the potential to investigate dynamic starvation conditions in CHO cell cultivations.

Finally, the third publication in Chapter 6 extends the industrial-scale investigations with additional LB LES for a different impeller setup (Fig. 3.2 c)). This study examines the accuracy with which LB LES reproduce experimental LSP trajectories and highlights key differences between LSPs and massless tracer particles, where the latter represent microbial

Thesis outline

cells during cultivation. The availability of data on the system's ergodicity during sampling is critically discussed, along with the representation of the LSP's center of mass in the numerical simulations, and the consistency of mixing heterogeneities across experimental and numerical approaches. Moreover, the study elaborates on the spatial scale at which LSPs resolve cell lifelines and how the Stokes number aids in characterizing the STR and critically evaluating the LSP design.

In the conclusion and outlook in Chapter 7, the main contributions of the thesis are summarized by revisiting the three fundamental RQs and outlining the key findings from experimental and numerical studies. It highlights the strengths and limitations of LSPs for lifeline analysis, and assesses the predictive power of simulation tools across scales. The chapter concludes with a discussion of the remaining challenges and future directions for advancing both sensor technology and simulation approaches.

Chapter 4

Lagrangian sensors in a stirred tank reactor: Comparing trajectories from 4D-Particle Tracking Velocimetry and Lattice-Boltzmann simulations

This chapter reproduces the publication: Hofmann, S., Weiland, C., Fitschen, J., von Kameke, A., Hoffmann, M., Schlüter, M., 2022. Lagrangian sensors in a stirred tank reactor: Comparing trajectories from 4D-Particle Tracking Velocimetry and Lattice-Boltzmann simulations. Chemical Engineering Journal 449, 137549 [Hof22b]. Utilized data and mathematical analyses can be accessed at the DaRUS repository [Hof22a] or in the Gitlab repository [Hof25c].

Lifelines on laboratory scale and in Lattice-Boltzmann simulations

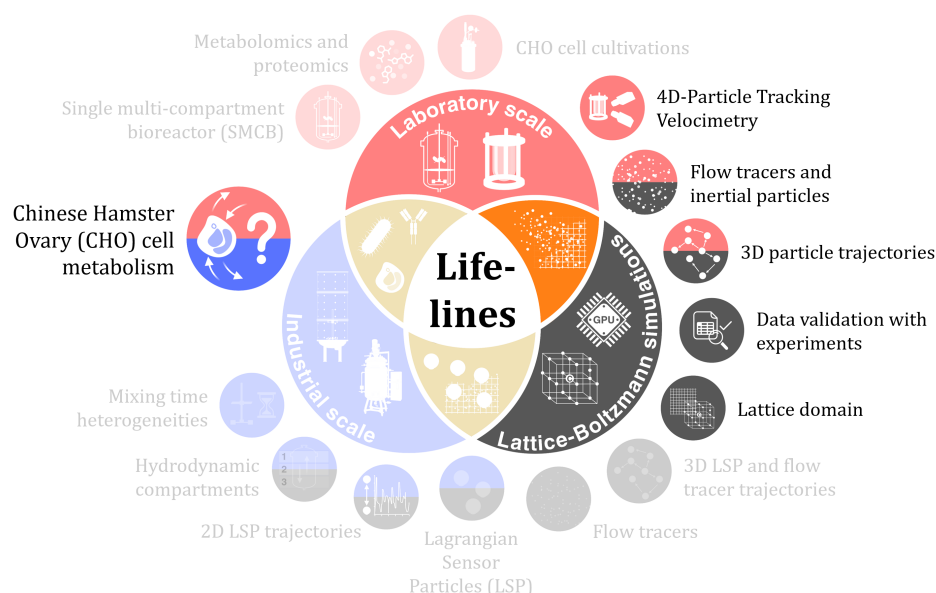


Figure 4.1: Figurative representation of the two investigation frameworks of the first publication: laboratory scale and Lattice-Boltzmann simulations

The first peer-reviewed publication elaborates on the investigation frameworks "laboratory-scale" experiments and "Lattice-Boltzmann simulations" (Fig. 4.1). The initial step is to characterize the physical behavior of particles with defined sizes and densities in a 3 L stirred tank reactor (STR), and to validate the corresponding Lattice-Boltzmann large eddy simulations (LB LES) using experimental time-resolved 3D particle trajectories. In this context, inertial particles of varying sizes are analyzed with respect to their ability to follow the flow. These particles serve as downsized analogues of Lagrangian Sensor Particles (LSP), which are applied on industrial scale in the upcoming publications.

Moreover, it is elaborated on how particle physics and their statistical evaluation can serve as a tool for assessing which particle sizes follow the flow and how these findings can be scaled up to a 15,000 L bioreactor. It is investigated what the requirements are for calculating the Stokes number based on 3D particle data, the associated limitations, and the suitability of this metric for quantifying and comparing flow-following behavior. Additionally, Eulerian and Lagrangian evaluation approaches are compared, and the mathematical analyses developed are prepared for application in industrial-scale trials. Ultimately, the study assesses whether the employed LB LES can be validated and whether they allow for reliable predictions to guide future LSP experiments on industrial scale.



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Lagrangian sensors in a stirred tank reactor: Comparing trajectories from 4D-Particle Tracking Velocimetry and Lattice-Boltzmann simulations

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ABSTRACT

In this study, three-dimensional flow measurements by means of 4D-Particle Tracking Velocimetry (4D-PTV) are carried out in a laboratory-scale 3 L stirred tank reactor in order to investigate the flow-following behavior of two different inertial particle types, Polyethylene (PE) particles and alginate beads, at different impeller frequencies. Applied particles mimic Lagrangian sensor particles, which are intended to determine process parameters such as oxygen concentration at their corresponding position inside a bioreactor. Accompanying Lattice-Boltzmann Large Eddy Simulations (LB LES) provide additional information about the fluid flow and the difference in the trajectories between inertial and non-inertial particles. The data acquired from LB LES is validated with the experimental data by means of a Lagrangian and a Eulerian approach. In their tail, the probability distributions show higher Lagrangian velocities and accelerations for 4D-PTV data compared to LB LES data. Time-averaged Eulerian data is utilized to determine particle Reynolds numbers lower than 200. The Stokes number distributions show 10-fold higher values for the alginate beads than for PE particles, however, both particle types do not sufficiently meet the criterion of a flow-following Stokes number of $St \leq 0.01$. Generally, time-averaged results from LB LES are in good accordance to the 4D-PTV data. From the LB LES, a theoretical, maximum particle diameter of approx. 20 μm is determined, which meets the criterion of $St \leq 0.01$ throughout the reactor. This result implies that with current sensor particle technology it is not possible to meet the flow-following behavior and depict the lifelines of cells during a cultivation process. Therefore, further research is necessary to understand particle trajectories and to translate them into lifelines of cells.

1. Introduction

In terms of biotechnology and cultivation of microorganisms, sensors are ubiquitously used in bioreactors in order to track multiple parameters like temperature, pressure, pH, dissolved oxygen concentration, cell density or conductivity [2–4]. Furthermore, the growing complexity of antibody or vaccine fabrication and a constantly increasing demand of sustainable production pathways requires precise monitoring and control. However, static probes are only able to represent a single-point measurement. Their measured values have to be taken into account for the entire system or at least for the respective volume fraction. Therefore, spatial gradients correspond to their number and mounting position. Specifically, the occurrence of compartments [5,6] may prevent reaching maximum yield and product titer due to the fact that cells experience transient culture conditions [7]. This widely reported phenomenon of occurring metabolic regimes [8] in a stirred tank reactor (STR) arises mainly in large-scale fermentors

with the capacity of up to 54 000 L [9] and affects multiple kinds of microorganisms as fungi [10], bacteria [11] and mammalian cell cultures [12–14]. Since cellular responses on industrial scale cannot be predicted experimentally *a priori*, considerable research has been devoted to Computational Fluid Dynamics (CFD) studies [6,15–20] to understand the mixing process in STRs. However, considering cell-cell and cell-bioreactor interactions, CFD simulation is not able to represent the entire complex cell metabolisms like the increase in respiratory activity, the accumulation of byproducts or even resulting phenotypic changes [7,8]. This is the reason why significant experimental work is still required to validate and optimize process behavior [21–23].

Due to the lack of optical access into such a production scale fermentor several research groups [1,24–32] devoted their focus on developing an alternative approach in order to get insight into either a bioreactor or generally a process with complex hydrodynamic properties. By means of applying and merging multiple sensor systems

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Lagrangian sensors in a stirred tank reactor: Comparing trajectories from 4D-Particle Tracking Velocimetry and Lattice-Boltzmann simulations

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Nomenclature			
Arabic symbols			
F_{ext}	External forces	r	Radial position in reactor
u	Fluid velocity	R_d	Ratio of particle diameter/reactor diameter
u_{ideal}	Velocity of simulated, non-inertial particles	Re_p	Particle Reynolds number
u_{inertial}	Velocity of simulated inertial particles	Re_{STR}	Global Reynolds number estimation for an STR
x	Position	S_p	Surface area of particle
a	Acceleration	S_{sphere}	Surface area of a sphere
A_p	Projected area of particle	St	Stokes number
Ar	Archimedes number	St_{MF}	Stokes number of the mean flow
b	Rushton Turbine single blade width	St_{mod}	Stokes number calculated by means of alternative τ_f by [1]
c	Baffle-reactor wall distance	$St_{\text{Re,alg}}$	Stokes number for alginate beads based on Eq. (2) and Re_{STR}
c_D	Drag coefficient	$St_{\text{Re,PE}}$	Stokes number for PE particles based on Eq. (2) and Re_{STR}
$c_{D,\text{corr}}$	Correction for drag coefficient	t	Time
Co	Courant number	u	Velocity of the fluid
d_1	Tank diameter	u_0	Root mean-square fluid velocity
d_2	Impeller diameter	u_p	Velocity of the particle
d_3	Rushton Turbine inner disc diameter	u_{tip}	Impeller tip speed
$d_{32,\text{alg}}$	Sauter diameter of alginate beads	V	Reactor working volume
d_{alg}	Alginate bead particle diameter	v_{mag}	Velocity magnitude
d_{nozzle}	Jet cutter nozzle diameter	V_p	Volume of particles
d_{PE}	PE particle diameter	w	Semi-minor axis of a spheroid
d_p	Particle diameter	x	Position in x direction on a Cartesian grid
e	Eccentricity of an ellipsoid	y	Position in y direction on a Cartesian grid
f	Particle distribution function	z	Position in z direction on a Cartesian grid
f^{eq}	Equilibrium particle distribution density	Greek symbols	
F_B	Buoyancy force	Δ	Difference or uncertainty
F_D	Drag force	ϵ_{loc}	Local energy dissipation rate
F_{vertical}	Vertical force	η	Dynamic viscosity
F_W	Weight force	γ	Surface tension
g	Gravitational acceleration	Λ	Turbulent length scale
h_1	Reactor filling height	λ	Kolmogorov length scale
h_2	Impeller bottom clearance	λ_{gly}	Wavelength for refraction index measurement of glycerol
h_3	Impeller spacing	ν	Kinematic viscosity
h_4	Rushton Turbine blade height	Ω	Collision parameter
k	Particle radius	$\bar{\epsilon}$	Mean energy dissipation rate
L	Characteristic flow length scale	$\Phi_{V,\text{alg}}$	Volume fraction of Alginate beads in working volume
L_{MF}	Chosen characteristic length scale for the mean fluid flow	$\Phi_{V,\text{PE}}$	Volume fraction of PE particles in working volume
L_{STR}	Characteristic length scale based on $0.5(d_1 - d_2)$	ψ_{alg}	Sphericity of alginate beads
L_E	Taylor scale	ρ	Density
m	Mass	ρ_{alg}	Alginate bead density
m_p	Mass of particle	ρ_f	Fluid density
n	Concurrent repetitions of trials	ρ_{LB}	Lattice-Boltzmann density
n_2	Impeller speed/Jet cutter speed	ρ_{PE}	PE particle density
n_s	Refraction index	ρ_p	Particle density
Ne	Newton number/Power number		
P	Power input		
P/V_{global}	Global power per volume input		
q	Semi-major axis of a spheroid		

into one mobile, encapsulated, free-floating sensor particle their common goal is to investigate and eventually understand gradients and instantaneous 3D profiles in a highly turbulent regime.

Despite of the sensor's capabilities to measure real-time process conditions and be free-floating, the most essential characteristic of

such a sensor particle is, however, to follow the flow in a sense to depict the trajectory of a microorganism appropriately. In this case, the term *lifeline* was established, which implies to focus on "individual cells in space and time" [33] or "represent cellular data as a function of time" [8]. This may reveal information for a thorough statistical analysis of microorganisms with respect to regime changes. One important prerequisite hereby is to characterize the flow behavior of

σ	Angle for vertical particle selection cut throughout reactor
τ	Relaxation time
τ_f	Time characteristic of the flow field
τ_p	Particle relaxation time
φ	Angle
ζ	Velocity of particles for the LBM
Abbreviations	
200LX	Grid spacing for Lattice-Boltzmann simulation
4D-PTV	4-dimensional Particle Tracking Velocimetry
alg	Alginate
CFD	Computational Fluid Dynamics
exp	Experimentally
LBM	Lattice-Boltzmann-Method
LED	Light Emitting Diode
OTF	Optical Transfer Function
PE	Polyethylene
PIV	Particle Imaging Velocimetry
ppp	Particles per pixel
rms	Root mean square
sim	Numerically
STB	Shake-the-Box
STR	Stirred Tank Reactor
Tomo-PIV	Tomographic-Particle Imaging Velocimetry
VSC	Volume Self Calibration

such an inertial sensor particle. Its shape, size and density are the foremost important parameters to determine the inertia with respect to the turbulent surrounding carrier flow. An underlying reduction of the root-mean-squared particle acceleration, meaning a response time delay gives evidence that a sensor does not follow the flow [34]. Due to current technological restrictions in battery size and miniaturization of Printed Circuit Boards (PCB) the sensors are attached to, the minimum size is predetermined, and thus the flow-following behavior in the respective flow field is at risk. Therefore, it is crucial to gain spatio-temporal information of sensor devices in large-scale bioreactors.

Although there are multiple ways to visualize particle flow fields in stirred tanks [35], for the purpose of acquiring velocity fields or even 3-dimensional, time-resolved trajectories of turbulent particle-laden flows in a lab-scale STR, approaches as Particle Imaging Velocimetry (PIV) [36–38] and Particle Tracking Velocimetry (PTV) [16,38–42] are the most precise state-of-the-art technologies. In 2016, a major development of the latter has been introduced by Schanz et al. The *Shake-the-Box* (STB) approach for Lagrangian particle tracking at high particle image densities [43,44] is a method, which accounts for highly resolved spatial and temporal particle tracks for seeding densities of up to 0.125 particles per pixel (ppp) with a setup of four or more cameras [44,45].

Due to its manifold capabilities, the 4D-PTV approach – in combination with STB – shows the most promising results to examine particle tracks in a 3 L STR more in depth. Two particle types with different sizes and densities are examined to meet the necessity to understand the acting inertial forces and the overall flow behavior. One flow-following characterization measure, which captures both previously stated values is the Stokes number St . It is defined as the ratio of (1) the particle momentum response time τ_p derived from the viscous drag term of the particle equation of motion to (2) the corresponding time scale of the carrier fluid τ_f [46]. Previous publications have found either experimentally or based on numerical simulations an increasing difference in

acceleration statistics contrasting large (26 times Kolmogorov length scale λ) [47] or heavy [34] inertial particles and fluid, respectively, in case of $St > 10^{-2}$ [48]. Further details about local Stokes numbers and the motion of large, density-matched or small, but heavy particles and their trajectories within a STR, however, have not been studied experimentally.

In this study, Lagrangian particles are tracked in a baffled 3 L STR using the 4D-PTV technique. This is the first experiment to provide not only detailed spatially resolved Lagrangian velocity but also acceleration data of inertial particles of different sizes and weights in a turbulent regime – inside the confined space of the STR. Accompanying Lattice-Boltzmann simulations in a numerical twin of the setup are validated by the experimental data and used for further fluid velocity and acceleration analyses. Therefore, the transient and instantaneous Lagrangian particle tracks of both experiments and numerical simulations are analyzed and discussed with respect to Lagrangian velocity and acceleration statistics. In order to estimate the flow-following behavior of inertial particles in the experiment the time-averaged Eulerian velocities are utilized to calculate the particle Reynolds numbers and the resulting Stokes numbers throughout the reactor. Numerical results are further investigated regarding spatially and time-resolved particle and associated fluid velocity data. Finally, a theoretical, spatially resolved, maximum particle diameter throughout the STR depicts the Lagrangian sensor size magnitude needed to accomplish the specification to be flow-following.

2. Material and methods

In this section, first the 3 L STR, which is used for both experiments and simulations is presented. Parameters and handling of the two particle types are described as well as the procedure of the 4D-PTV measurements with high-speed cameras, the image pre-processing with a custom-made Matlab code and the subsequent trajectory evaluation by means of the software module STB. Afterwards, the Stokes number for the corresponding particle in a fluid flow is derived and necessary prerequisites as the particle Reynolds number and the Archimedes number are disclosed. Finally, essentials and model details for the numerical simulations are stated together with a description of its procedure to compare resulting particle data with herein mentioned experiments.

2.1. Stirred tank reactor setup

In this study a 3 L STR with a working volume of $V = 2.8$ L is used for both 4D-PTV measurements and numerical simulations. The vessel, with a bottom shape of a korbogen head, has an inner diameter of $d_1 = 0.13$ m and is equipped with two Rushton turbines ($d_2 = 0.036$ m) and three baffles at $\sigma = 120^\circ$ displacement. It is filled with demineralized water at room temperature at 20°C ($\rho_f = 998.2$ kg m $^{-3}$, $\eta = 1.0016$ mPas, $\gamma = 0.0728$ N m $^{-1}$) up to a filling height of $h_1 = 0.228$ m. All further dimensions are depicted in Fig. 1 and correspond to those previously published [16]. In order to compensate the refraction of the borosilicate glass tank ($n_s = 1.470 - 1.474$ [49]), for the experimental investigations an octagonal outer container is filled with glycerol ($n_s = 1.471 \pm 0.4 \cdot 10^{-3}$, $\lambda_{\text{gly}} = 589.3$ nm (Abbemat WR MW, Anton Paar)) at the same temperature of the containing demineralized water. The outer container and the internally installed baffles are made of acrylic glass ($n_s = 1.486 - 1.491$ [49]). The material of the shaft and the two Rushton turbines is stainless steel, painted with a flat, black varnish. Used stirring speeds (ViscoPakt-rheo-16, HiTec Zang GmbH) are 252 rpm (corresponds to a tip speed of $u_{\text{tip}} = 0.48$ m s $^{-1}$) and 450 rpm ($u_{\text{tip}} = 0.85$ m s $^{-1}$). If a Newton number of 7.5 [16] for both Rushton turbines is taken into account these speeds correspond to a global volumetric power input P/V_{global} of 12 W m $^{-3}$ and 68 W m $^{-3}$, respectively. The stirrer Reynolds number for this system, described as

$$Re_{\text{STR}} = \frac{\rho_f n_2 d_2^2}{\eta}, \quad (1)$$

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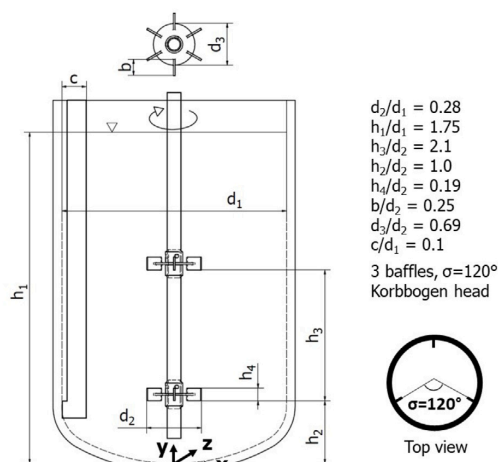


Fig. 1. Dimensions of the 3 L STR used for both experimental 4D-PTV measurements and numerical simulations, with $d_1 = 130$ mm and $d_2 = 36$ mm.

results in $5.4 \cdot 10^3$ and $9.7 \cdot 10^3$, respectively, where ρ_f is the fluid density, n_2 is the impeller speed, d_2 the impeller diameter and η the dynamic viscosity.

2.2. Experimental setup for 4D-Particle Tracking Velocimetry (4D-PTV)

2.2.1. Fluorescent particles

Inertial particles with similar preconditions as instrumented particle sensors in production-scale fermentors and their trajectory are considered and analyzed for the first time by means of 4D-PTV. According to Zlokarnik, the largest eddy present, in which the sensor moves, is of the magnitude of the turbulence generator [50]. That is the reason why this study chooses a scale-down approach considering a theoretical, spherical, mobile micro-sensor with a diameter of $d_p = 0.013$ m, which can be applied to a production-scale bioreactor with an impeller diameter of $d_2 = 0.66$ m. Hence, the particle-impeller diameter ratio $R_d = d_p/d_2$ is 0.02. To illustrate this ratio in a more controllable environment like a 3 L lab-scale bioreactor with an impeller diameter of $d_2 = 0.036$ m, the particle diameter can be accordingly reduced with keeping R_d constant, resulting in a particle size of approx. 0.71 mm.

In this work, two different types of particles are used to investigate the Lagrangian particle motion in the 3 L bioreactor. One are fluorescent polyethylene (PE) particles with the size of $d_{PE} = 150 - 180$ μm and a density of $\rho_{PE} = 1000$ kg m^{-3} (Cospheric LLC, Santa Barbara, CA, USA). The others are custom-made calcium alginate beads (see Appendix B) with a Sauter mean diameter of $d_{32, \text{alg}} = 732$ μm , a density of $\rho_{\text{alg}} = (1023.6 \pm 1.1)$ kg m^{-3} and a sphericity of $\psi_{\text{alg}} = 0.998 \pm 0.004$ (see Appendix C). Here, the bead size is measured with the software ImageJ (Version 1.53a) and the density by means of a pycnometer (Paul Marienfeld GmbH & Co. KG, Germany).

The total number of particles dispersed in the STR is approx. 17 000 for the PE particles and 3800 for the alginate beads. This matches particle seeding densities of 0.02 particles per pixel (ppp) and 0.005 ppp, respectively. For a successful Optical Transfer Function (OTF) calibration, these values are taken based on publications by Schanz et al. [44,51] and Wieneke et al. [43]. The total number of particles is an estimation from multiple image sections (see Fig. 3(d) and (e)). This results in a mean particle distance of $5.5 \cdot 10^{-3}$ m and $9.0 \cdot 10^{-3}$ m, corresponding to volume fractions of $\Phi_{V, PE} = 0.02 \cdot 10^{-3}$ and $\Phi_{V, \text{alg}} = 0.29 \cdot 10^{-3}$, respectively.

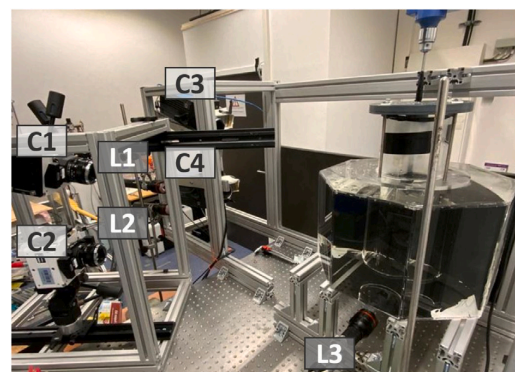


Fig. 2. Experimental setup at the Institute of Multiphase Flows at Hamburg University of Technology. C1, C2, C3 and C4 mark the four high-speed cameras used for the measurements of the Lagrangian tracks. L1, L2 and L3 are the three high-power LED's for illuminating the respective particles in the tank.

2.2.2. Preparation and conduct of measurements

Shown in Fig. 2, two PHANTOM VEO 640 L (C1 and C2 in the front) and two pco.dimax HS2 (C3 and C4 in the back) are used to record the particles inside the reactor, whereas both camera sets have a horizontal offset of 45° towards each other and the two identical cameras are mounted at an angle of 30° with respect to the vertical direction. The camera pixel sizes are 10 μm and 11 μm , respectively, such that a spatial resolution of approximately 150 $\mu\text{m}/\text{pixel}$ is reached at a 12-bit depth. For a proper optical access, Scheimpflug adapters, lenses by Carl Zeiss (Macroplanar, 50 mm f/8.0) and 500 nm long-pass filters (Edmund Optics Ltd., York, UK) that prevent recording the scattered light from the light sources are used.

Prior recording, the 3 L reactor is calibrated by means of a calibration target, which has a width of 120 mm and on its surface a grid of 24×41 equidistantly spaced dots of 1 mm in diameter and 5 mm distance towards each other to define the horizontal x- and the vertical y-axis. To fit the reactor bottom shape the target is adjusted to the characteristic korbogen head (see Fig. 3(a)). Eventually, to define the three-dimensional Cartesian grid, the z-axis is added by shifting the calibration target in seven equally spaced distances (6.2 mm each) to reach an overall displacement path of 37.2 mm in depth. The calibration is a prerequisite to start the Volume Self Calibration (VSC), which is a particle-based correction of the calibration function [44] and calculates the Optical Transfer Function (OTF) to improve the volume reconstruction of the 3D position-dependent particle image shape [51]. It is worthwhile to note that gathered Lagrangian data utilize the identical Cartesian reference system as the eventually calculated Eulerian data.

The measurements are carried out with approx. 17 000 PE particles and 3800 alginate beads dispersed in the STR (see Fig. 3b and c). That corresponds to a particle seeding density of 0.02 ppp and 0.005 ppp and a corresponding particle image diameter of approx. 3–4 px and 5–6 px, respectively. With these loading densities particle interactions are negligible. For the lighting, three high-power LED's (OSRAM, device mounted in-house in flashlight housings, see Fig. 2, marked as L1, L2 and L3) account for the excitation of the particle's fluorescence with an exposure time for each image of 400 μs and 600 μs , respectively. A programmable timing unit from LaVision synchronizes both the LED's and the camera signals reaching a temporal resolution of 2381 μs and 1905 μs (420 Hz and 525 Hz). Due to the maximum recording capacity of 3000 images, the overall recording time is 7.14 s and 5.71 s, respectively.

Eventual calculation of Lagrangian particle tracks is achieved by means of the commercial software FlowMaster from LaVision

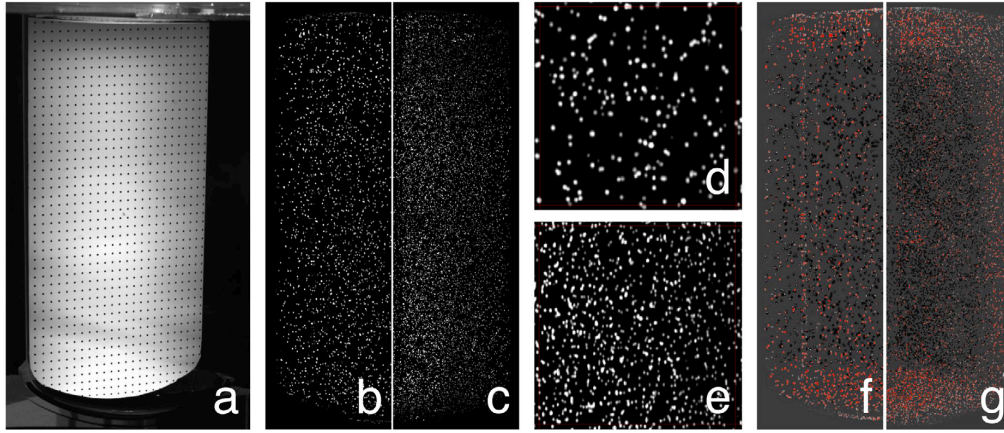


Fig. 3. Sample images from calibration and recording of respective particles in a 3 L reactor. (a) Calibration target inserted from top into the reactor. Processed sample image of the half reactor of (b) alginate particles and (c) PE particles. Section image with the size of 200×200 pixel of (d) alginate particles and (e) PE particles. Residual images of one single camera depict tracked (f) alginate beads and (g) PE particles (local minima), which enter the next consecutive “shaking step” and residuals (local maxima as red dots) as new particles.

(DaVis 10.1.2.73698, Göttingen, Germany). One crucial intermediate step is the image pre-processing, which is conducted in a custom-made Matlab code [52]. Here, the background signal is subtracted prior applying a high-pass (sharpening) and low-pass (denoising) digital Butterworth filter subsequently in order to improve the particle shape and lower the background signal for an optimal image preparation to use STB. After several iterations of shaking in STB, the residuals of tracked particles vanish in the residual image of one single camera, whereas new particles remain on the respective image. Fig. 3 shows the high number of residual (f) alginate beads and (g) PE particles particularly in the reactor bottom region.

2.3. Calculation of non-dimensional numbers: Stokes-, particle Reynolds- and Archimedes number

As one flow-following appraisal criteria of inertial particles is the Stokes number, which is defined as

$$St = \frac{\tau_p}{\tau_f} \quad (2)$$

It compares the response time of a particle τ_p to the temporal scale of changes in the Lagrangian velocity of the surrounding fluid τ_f . The origin of the single terms of the Stokes number is the equation of motion given by Maxey and Riley [53], stating all important forces for a single particle in motion. Here, only the net body force

$$F_{\text{vertical}} = (\rho_p - \rho_f)gV_p \quad (3)$$

and the drag force F_D acting on the particle with

$$F_D = 0.5\rho_f c_D \|u - u_p\|_2 (u - u_p) A_p \quad (4)$$

are considered, where $\|\cdot\|_2$ indicates the 2-norm of a vector. According to Newton's second law, with respect to particles with a constant mass m_p and in terms of their acceleration a the total force F can therefore be derived as

$$m_p \frac{du_p}{dt} = 0.5\rho_f c_D \|u - u_p\|_2 (u - u_p) A_p + (\rho_p - \rho_f)gV_p. \quad (5)$$

This is a very simplified version of the equation of motion in stationary flows, in which non-stationary forces, Basset history forces, Faxen term and the added mass term are neglected [54]. Furthermore, by introducing the Reynolds number for particles in a fluid flow Re_p as

$$Re_p = \frac{\rho_f d_p \|u - u_p\|_2}{\eta} \quad (6)$$

the expression

$$\frac{du_p}{dt} = \underbrace{\left(\frac{18\eta}{\rho_p d_p^2} \right) \left(\frac{c_D Re_p}{24} \right)}_{\frac{1}{\tau_p}} (u - u_p) + \left(\frac{\rho_p - \rho_f}{\rho_f} \right) g \quad (7)$$

is obtained. By the solution of this ODE, as detailed in Crowe et al. [46] the coefficient of the first term on the right hand side is the inverse of the particle momentum response time τ_p , also called particle relaxation time. This expression allows an estimate regarding the particle response to the fluid flow field u . Furthermore, if Stokes drag applies the expression $c_D Re_p/24$ approaches unity [55]. However, in this study inertial particles are investigated, which is why the drag coefficient as a non-Stokesian correction factor has to be taken into account as

$$c_D = \frac{24}{Re_p} (1 + 0.15 Re_p^{0.687}), \quad (8)$$

which is true for $1 \leq Re_p \leq 800$ [56]. The volume fractions of $\Phi_{V, PE} = 0.02 \cdot 10^{-3}$ and $\Phi_{V, alg} = 0.29 \cdot 10^{-3}$ are low enough not to have an influence neither on c_D [57] nor on acceleration statistics [58]. In this study, no correction factor for the drag coefficient is taken into account (see Appendix D). This concludes eventually to

$$\tau_p = \frac{4}{3} \frac{\rho_p d_p^2}{\eta c_D Re_p}. \quad (9)$$

The time characteristic of the flow field τ_f (eddy turnover time) is the ratio of the characteristic fluid flow length scale L and the velocity of the carrier fluid u and can be stated as

$$\tau_f = \frac{L}{\|u\|_2}. \quad (10)$$

With the general expression of the Reynolds number for the fluid Re_f as

$$Re_f = \frac{\rho \|u\|_2 L}{\eta} \Leftrightarrow \|u\|_2 = \frac{Re_f \eta}{\rho L}, \quad (11)$$

Eq. (10) can be stated in different forms as

$$\tau_f = \frac{L^2 \rho_f}{Re_f \eta} \quad (12)$$

$$\tau_f = \frac{d_2}{v_{\text{tip}}}, \quad (13)$$

whereas Eq. (13) is a special form introduced by Reinecke et al. [1], considering the impeller diameter d_2 and the impeller tip velocity v_{tip} .

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For non-inertial particles, Ouellette et al. [48] defined the Stokes number based on the particle radius k and the characteristic flow length scale L (Eq. (10)) as

$$St = \frac{2}{9} \frac{\rho_p}{\rho_f} \left(\frac{k}{L} \right)^2 Re_f. \quad (14)$$

However, for inertial particles that are not in the Stokes regime like in this study, the Stokes number is slightly different. Based on Eq. (9), substituting Re_p by Eq. (6) and the eddy turnover time from Eq. (10) this concludes to

$$St_{MF} = \frac{4}{3} \frac{\rho_p}{\rho_f} \frac{d_p}{\|u - u_p\|_2} \frac{\|u\|_2}{L} c_D, \quad (15)$$

and considers the corrected response time due to non-Stokesian drag [54]. According to Huilier, care has to be taken to evaluate the correct Stokes number, and thus here the Stokes number of the mean flow St_{MF} is investigated. In this case, “MF” is the short form for “mean flow” and contemplates the flow-following behavior of particles regarding the mean fluid flow (see Eq. (10)) within its respectively defined characteristic flow length scale L . However, differing system parameters, relaxation times τ_p and τ_f and especially individual definitions of the characteristic length make it nearly impossible to compare particle-laden flows in turbulent regimes. Generally, L can be defined as the Taylor scale L_E [54], like

$$L_E = u_0 \sqrt{\frac{15\nu}{\bar{\epsilon}}}, \quad (16)$$

in which u_0 denotes the root-mean-squared (rms) fluid velocity, which is gained from the simulations as stated above, ν is the kinematic viscosity and $\bar{\epsilon}$ denotes the mean energy dissipation rate, as calculated in the Eqs. (35) and (36). At this scale, eddies start to become affected by viscous dissipation. The results mark $L_{E,252 \text{ rpm}} = 2.9 \text{ mm}$ and $L_{E,450 \text{ rpm}} = 2.2 \text{ mm}$. In this context, Ochieng et al. refer to a turbulent length scale of $\Lambda = 0.1 d_s$, which would result to 3.6 mm [59]. However, for this study, a characteristic flow length scale of $L_{MF} = 1 \text{ mm}$ is chosen, which is a compromise between the Taylor scale L_E and the turbulent length scale Λ on the one hand and the distance between lattice points for the numerical simulations ($\Delta x = 0.646 \text{ mm}$) and the alginate bead size ($d_{32,alg} = 0.732 \text{ mm}$) on the other hand in order to receive a highest possible resolution.

Another important non-dimensional number describing the settling phenomena of a particle is the Archimedes number Ar . It is defined as the ratio of buoyancy force to frictional force as

$$Ar = \frac{\rho_f(\rho_p - \rho_f)gd_p^3}{\eta^2}. \quad (17)$$

Here, ρ_f and ρ_p are the density of the fluid and the particle, respectively, g the gravitational acceleration, d_p the diameter of the particle and η denotes the dynamic viscosity of the fluid. For the PE particles with a density of $\rho_p = 1000 \text{ kg m}^{-3}$ an Archimedes number of $Ar = 0.1$ is calculated. Moreover, for alginate beads $Ar = 98.8$, meaning the larger the particle the larger the influence of buoyant forces, despite of a very small density difference of $\Delta\rho_p = 24 \text{ kg m}^{-3}$. Consequently, these values suggest a homogeneous suspension due to $Ar \lesssim 100$, and therefore the drag force dominates over the gravitational force on the particles [60].

2.4. Lattice-Boltzmann simulations

In the numerical simulations the same geometry as in the experimental setup was used. The transient simulations were carried out with the program M-Star CFD 3.0.19 (M-Star Simulations, LLC, Ellicott City, MD, USA), which solves the Boltzmann-equation

$$\frac{\partial f}{\partial t} + \zeta^T \nabla_x f + F_{ext}^T \nabla_\zeta f = \Omega(f) \quad (18)$$

for a set of particles numerically. Hereby are f the particle distribution function, ζ the velocity of these particles, F_{ext} external forces and Ω the

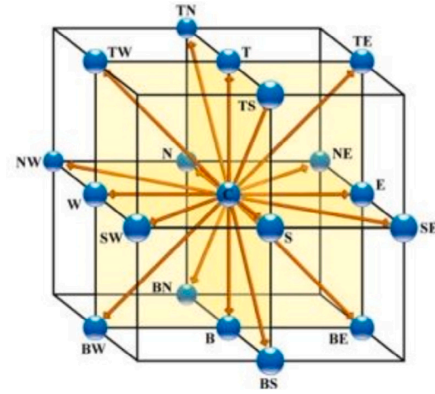


Fig. 4. The D3Q19 velocity basis, each particle can only assume any of these discrete velocities [62].

collision parameter. As collision parameter the Bhatnagar–Gross–Krook collision parameter

$$\Omega(f) = -\frac{1}{\tau} \cdot (f - f^{eq}) \quad (19)$$

with the relaxation time τ and the equilibrium particle distribution density f^{eq} was used [61]. For the discretization of the velocity space the D3Q19 velocity set (compare Fig. 4) and for the solution in time the forward Euler scheme was used to achieve the Lattice-Boltzmann-equation

$$f_i(x + c_i \Delta t, t + \Delta t) = f_i(x, t) + \Delta t \Omega_i(x, t). \quad (20)$$

For solid–fluid boundary conditions two kinds were used: static boundaries/surfaces and moving boundaries/surfaces. Static surfaces were modeled by means of the mid-way bounce-back approach. In the course of this approach the concerning static boundary is placed mid-way between the nearest lattice points and an additional set of ghost lattice points on the other side. The ghost lattice points reflect the occurring probability distribution back according to the chosen discretization scheme [62]. Moving surfaces are modeled using the immersed boundary method [63]. The moving surface is modeled as cloud of Lagrangian points which move continuously in space and time. The interaction between the surface and the fluid can be described by Newton’s second law and usage of the no-slip condition [62]. Macroscopic variables can be recovered by

$$\rho \equiv \int f(x, \zeta, t) d\zeta \approx \sum_i f_i \quad (21)$$

$$\rho u \equiv \int \zeta f(x, \zeta, t) d\zeta \approx \sum_i c_i f_i. \quad (22)$$

For simulations of turbulent flows a Large Eddy Simulation (LES) was conducted. To model the turbulent viscosity ν_T , the Smagorinsky–Lilly sub-grid scale model [64,65]

$$\nu_T = (C_S \cdot \Delta x)^2 \cdot \sqrt{2\bar{S}} : \bar{S} \quad (23)$$

was used with a Smagorinsky constant of $C_S = 0.1$ [57,66] and the resolved strain rate tensor $\bar{S}$. The Smagorinsky–Lilly sub-grid scale model is a widely used model. Despite its weaknesses for only considering dissipative energy transfer and utilizing empirical values, it is nevertheless easy to implement for a broad range of applications, where it delivers reasonable results [67].

To guarantee stability, the time step width was chosen to yield a Lattice-Boltzmann density of

$$0.95 \leq \rho_{LB} \leq 1.05. \quad (24)$$

For this purpose the Courant number has to satisfy the inequality

$$Co = \|u\|_2 \cdot \frac{\Delta t}{\Delta x} \leq 0.05 \quad (25)$$

as was recently shown by Kuschel et al. [16].

Since the apparatus geometry of this work matches the one used by Kuschel et al. [16], who conducted already a mesh study, no in-depth mesh study was carried out again. Similarly, 250 lattice points over the reactor diameter were chosen to achieve a sufficient accuracy for the velocity field of the continuous phase. To simulate particles on the other hand problems are likely to occur when particles with greater sizes than the lattice spacing are used. 250 lattice points yield a lattice spacing of $\Delta x = 0.52$ mm, which is high enough for the small particles (mimicking PE particles, compare chapter 2.2.1), but not for the ones mimicking alginate beads with a diameter of $d_p = 0.732$ mm. For these simulations a lattice spacing of $\Delta x = 0.646$ mm (equals 200 lattice points over the reactor diameter) is used. This value is a trade off between the accuracy of particle simulation and the accuracy of the simulation of the hydrodynamics of the continuous phase. In the following, the term “200LX” refers to this degree of discretization.

Three different types of particles were considered in the simulations: two kinds of inertial particles and ideally flow-following particles. The inertial particles had the parameters (I): $d_p = 180$ μm , $\rho_p = 1000$ kg m^{-3} and (II): $d_p = 732$ μm , $\rho_p = 1024$ kg m^{-3} mimicking PE particles and alginate beads, and thus denoted by the subscript “inertial” in the following. All types of particles move continuously in space. The path lines of the inertial particles are achieved via Newton’s second law

$$m_{\text{inertial}} \ddot{\mathbf{x}}_{\text{inertial}} = \sum_i \mathbf{F}_i, \text{ with } \mathbf{x}_{\text{inertial}}(t_0) = \mathbf{x}_{\text{inertial},0} \quad (26)$$

where the $\mathbf{F}_i$ are outer forces, namely the gravitational, buoyancy and drag force and the force due to the added mass and $\mathbf{x}_{\text{inertial},0}$ is the position of the particle at time t_0 [68]. The pathlines of the non-inertial particles can be retrieved directly from the velocity field of the fluid via

$$\mathbf{x}_{\text{ideal}}(t) = \int_{t_0}^t \mathbf{u}(\mathbf{x}_{\text{ideal}}(t'), t') dt' + \mathbf{x}_{\text{ideal}}(t_0) \quad (27)$$

since these particles are perfectly flow-following. If both types of particles are released at the same point in space and time, it can be stated whether or not the inertial particles are following the flow. To estimate how much the inertial particles deviate from the ideal ones the Euclidean distance

$$d(\mathbf{x}_{\text{inertial}}, \mathbf{x}_{\text{ideal}}) := \|\mathbf{x}_{\text{inertial}} - \mathbf{x}_{\text{ideal}}\|_2 \quad (28)$$

of both is calculated alike a relative dispersion. The metric (28) yields directly the deviation from an ideal particle and is a good indicator for the degree of the flow-following capability of particles. The data retrieved during the simulation of pathlines of particles gives not only insight into the position of these particles but also into the velocity of the particle and the velocity of the fluid at the very position of the observed particle. Additionally, another value to evaluate the flow-following capability of inertial particles is estimated. With the velocity of the inertial particle $\mathbf{u}_{\text{inertial}}$ and the velocity of the fluid $\mathbf{u}$ at a specific position $\mathbf{x}$ the angle φ between these two vectors can be calculated via [69]

$$\varphi = \arccos\left(\frac{\mathbf{u}_{\text{inertial}}^T \mathbf{u}}{\|\mathbf{u}_{\text{inertial}}\|_2 \cdot \|\mathbf{u}\|_2}\right). \quad (29)$$

The greater the angle φ is the lower the capability to follow the flow is. A perfectly flow-following particle would have an angle of $\varphi \equiv 0$ at any position. The angle is not the only value, which can be used to measure a difference in the Lagrangian velocities. The magnitude of these vectors can also differ from each other. For that purpose a similar metric like the one in Eq. (28) can be used. To account for the possibility of opposite directions of the two vectors this metric (28) is corrected by the sign function and yields

$$\tilde{d}(\mathbf{u}_{\text{inertial}}, \mathbf{u}) := \text{sign}(\mathbf{u}_{\text{inertial}}^T \mathbf{u}) \cdot \|\mathbf{u}_{\text{inertial}} - \mathbf{u}\|_2, \quad (30)$$

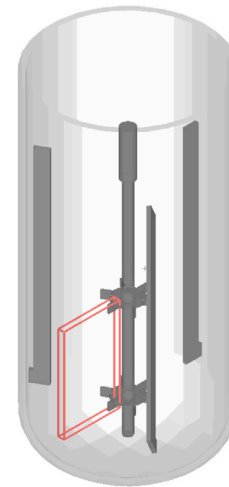


Fig. 5. Simulative setup, equivalent to experimental setup (Fig. 1), red box indicates starting position of particles.

where

$$\text{sign } x := \begin{cases} 1, & \text{for } x \geq 0 \\ -1, & \text{else.} \end{cases} \quad (31)$$

The simulations are carried out by letting the fluid velocity field evolve to a quasi-stationary state over $t_{\text{stat}} = 20$ s with a stirrer speed of either $n = 252$ rpm or $n = 450$ rpm. For the simulations performed to reveal the difference between ideal and inertial particles, the particles are added on a regular grid at t_{stat} . The grid of particles starts at point $\mathbf{x}_{\text{add}} = (-0.0025 \text{ m} \quad 0.037 \text{ m} \quad 0.01 \text{ m})^T$ with a displacement of $\Delta \mathbf{x}_p = (0.001 \text{ m} \quad 0.002 \text{ m} \quad 0.002 \text{ m})^T$ in between them and a total number of particles of $n_p = (5 \quad 42 \quad 25)^T$ in the respective dimensions. The borders of this grid can be seen in Fig. 5. These values are chosen to cover the area of interest, the volume between the stirrers and the reactor wall.

For the simulations, which launched to reveal the angle between the Lagrangian particle and the fluid velocity, particles are added on the same grid mentioned but evolved from the beginning of the simulation at time $t = 0$. For any other simulation the three particle sets are added in a randomized manner in the whole reactor, evolved over a time of t_{stat} . The recording of data was started at the time t_{stat} . In these cases 5000 particles of type (I) and 3800 of type (II) were added to approximately match the specifications of the experimental setup. For each simulation the recording of data was started at t_{stat} .

All simulations were carried out on a cluster with two AMD Epyc 7702 64 core processors and an Nvidia Tesla A100. With this setup, a typical simulation for this work took about one or two hours for a stirrer frequency of $n = 252$ rpm or $n = 450$ rpm, respectively.

3. Results and discussion

In this study, the flow-following behavior of two kinds of inertial particles is investigated experimentally and numerically alongside the particle’s trajectory inside an STR. PE particles ($d_{\text{PE}} = 150 - 180$ μm , $\rho_{\text{PE}} = 1000$ kg m^{-3}) and alginate beads ($d_{32,\text{alg}} = 732$ μm , $\rho_{\text{alg}} = (1023.6 \pm 1.1)$ kg m^{-3}) are applied in different measurement runs to the working volume of 2.8 L and mixed homogeneously with two Rushton turbines at two different mixing speeds of 252 rpm and 450 rpm. The experimental data is obtained by means of the 4D-PTV method. In the process, acquired Lagrangian data utilize the identical Cartesian

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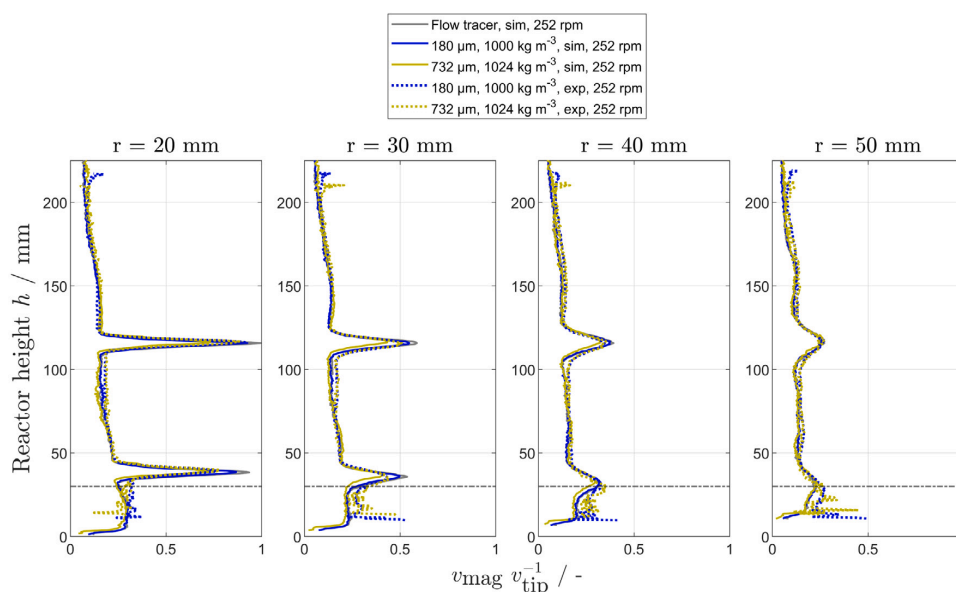


Fig. 6. Normalized velocity magnitude profiles over the reactor height (for 252 rpm) for four radial positions in the reactor, time-, axially- and azimuthally-averaged for a radial bin size of 4 mm and an axial bin size of 0.5 mm. Simulated (solid lines) and experimental results (dashed lines) are compared qualitatively. Gray dashed line at $y = 30$ mm depicts a cut-off for upcoming statistical evaluation due to high distortions in the reactor bottom.

reference system as the eventually calculated Eulerian data. To show the statistical relevance, details about the track length and the analysis of the number of tracks can be viewed in [Appendix A](#). Lagrangian and Eulerian results are statistically evaluated and compared with respect to velocity and acceleration data from the Lattice-Boltzmann simulations. Forces on the particles are derived from the experiments and evaluated as a 2D contour plot over the reactor radius and height. Subsequently, the Reynolds numbers of the particles are discussed referencing to two highly turbulent regions in the vicinity of the impeller, followed by an evaluation of the corresponding Stokes numbers of the mean flow St_{MF} . A 2D contour plot demonstrates prevailing zones of different flow-following behavior. For the purpose of calculating the particle Reynolds- and Stokes number, the respective time-, axially- and azimuthally-averaged experimental particle velocity u_p is subtracted by the undisturbed fluid flow velocity, received from the corresponding time-, axially- and azimuthally-averaged simulated velocity u of massless tracers in order to define the slip velocity in this study for the different types of finite-sized particles. Eventually, these results are qualitatively compared to solely numerical data to better understand the reasons for the differences between the velocity field of the observed particles u_p and the fluid u . This numerical study is accompanied by a comparison of inertial and non-inertial particles and their relative displacement while evolving in an STR. The results section closes with an estimation of a theoretical, maximum particle diameter, which is needed to obtain the “flow-following” criteria in the used STR.

3.1. Statistical evaluations of velocity and acceleration

Radially pumping Rushton turbines are widely used in all kinds of industries, where high mass transfer and high discharge rates are needed due to gassing or general chemical reactions. The predominant flow direction is from the impeller towards the vessel wall, where it deflects upwards and downwards. The subsequent formation of an upper and a lower toroidal vortex with opposing directions of rotation is a typical characteristic of this impeller type. In this subsection, the discharge flow region of the two impellers and intermediate regions are

scrutinized regarding their occurring velocity and acceleration. Four azimuthal slices through the reactor and further single-point statistics are used to demonstrate experimental data and compare it to numerical data. Henceforth, experimentally gained results are utilized to validate Lattice-Boltzmann simulations, which provides the opportunity to analyze flow field data and transient structures of the received Lagrangian particle tracks more in detail.

Fig. 6 shows the time-averaged velocity magnitude profiles of the experimental and simulated data. Each plot represents a certain azimuthal cut through the reactor (with $r \in \{20, 30, 40, 50\}$ mm and a radial bin size of 4 mm) along the reactor height (with a bin size of 0.5 mm), whereas the velocity is normalized by the tip speed of 0.47 m s^{-1} and respective cylindrical data are narrowed down to the radius by $r = \sqrt{x^2 + z^2}$. The data set *Flow tracer* depicts the simulated flow field of the fluid obtained by means of massless tracer particles. Experimental and simulative particle profiles show a very good qualitative agreement. Nevertheless, three general observations must be noted, as firstly, the simulated alginate beads (solid dark yellow line) show in the reactor bulk region persistently slightly lower velocity values compared to the experimental data (dark yellow dashed line), and secondly, both experimental data sets reveal a higher relative velocity in the lower suction zone of the upper impeller stage compared to all simulated data. The first aspect can be explained by the distance between each Lattice point alongside its respective Eulerian grid coordinate. With the grid meshing of 200LX and a corresponding distance between two points of 0.65 mm, this may directly have influence on the equation of motion for the alginate beads, which have a diameter of 0.732 mm, hence, are larger than the mesh (see Section 2.4). Therefore, the respective velocities may be debatable [54]. The second aspect may be due to obscured particles behind the shaft and, thus, a higher uncertainty in this region. Another effect is not clearly evident from the retrieved data.

Lastly, in the reactor bottom region the experimental data deviate strongly, which originates from high distortion despite of all measures taken for the geometric calibration. This is the reason why the gray dashed line refers to the bottom line at $y = 30$ mm, under which all information for the upcoming statistical single-point evaluations for

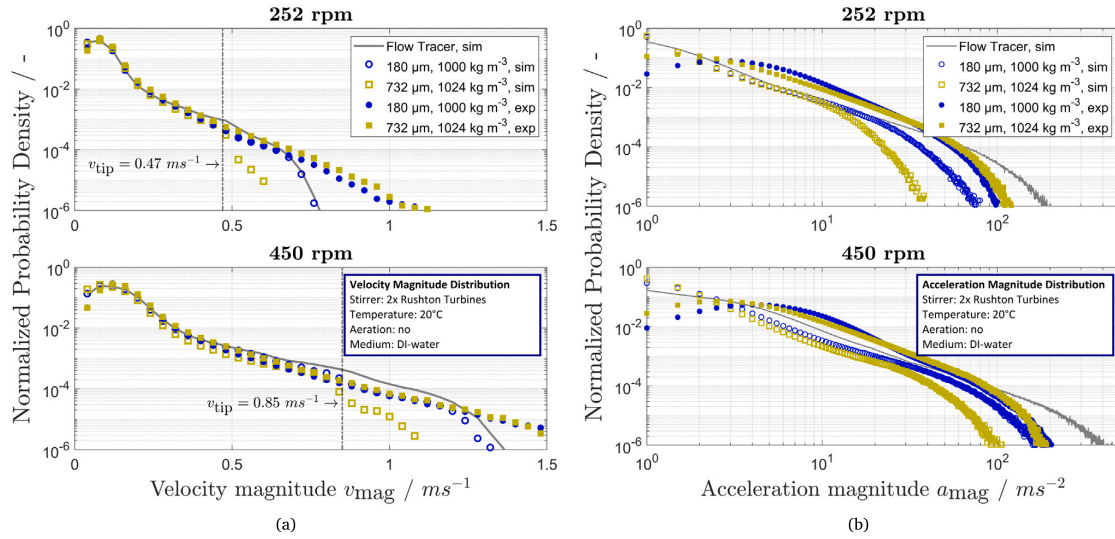


Fig. 7. Normalized probability density as a function of Lagrangian velocity and Lagrangian acceleration magnitudes, each at 252 rpm and 450 rpm for simulative (sim, hollow symbols) and experimental (exp, filled symbols) runs for PE particles (blue) and alginate beads (dark yellow). (a) PDF for the respective velocity magnitude with indication of the tip speeds. (b) PDF for the respective acceleration magnitude. In both data sets, the corresponding data under $y = 30$ mm is removed due to high distortions in the reactor bottom.

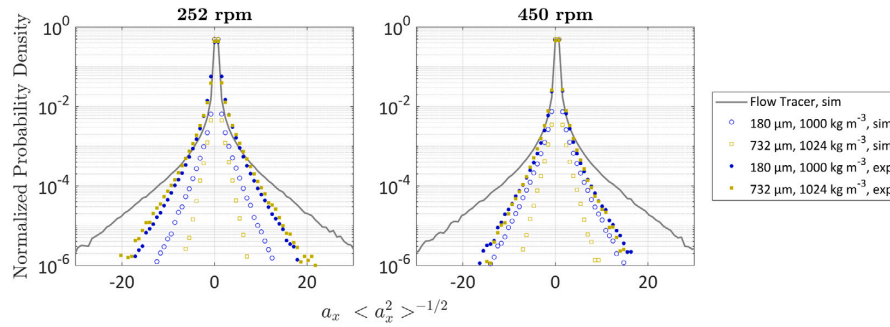


Fig. 8. Normalized PDFs of the Lagrangian particle accelerations a_x for 252 rpm (left) and 450 rpm (right). The accelerations are normalized by the rms value taken from the simulated, ideal fluid flow tracers (gray line). All data under $y = 30$ mm is removed due to high distortions in the reactor bottom.

both experimental and simulation data are removed. In tendency, it is to mention that all data seem to have an overall good agreement. All described observations can be seen similarly in the data set for 450 rpm (see Appendix E). Since depicted data plots in Fig. 6 show a very good qualitative agreement, each single particle type plot compared to the flow tracer plot can be found in Appendix F.

A more detailed statistical evaluation regarding all occurring Lagrangian velocities for the two impeller speeds is depicted in Fig. 7(a). The probability density of the Lagrangian velocity magnitude v_{mag} of the experimental and simulated data for both impeller speeds for all recorded data points are shown. It is evident that both trends for numerical (hollow symbols) and experimentally gathered data (filled symbols) show the occurrence of velocities higher than the respective tip speed, which is not seen in Fig. 6 because of time- and azimuthally-averaged velocity profiles. This issue was also addressed in Kuschel et al. [16]. The simulated massless flow tracer data (solid gray line) provides guidance when investigating inertial particles. The deviation between simulated and experimental data for low velocities may be explained by the obscured shaft and baffle regions, as described above. Furthermore, in case of simulations, there is no occurrence of faster particle velocities

compared to the ideal fluid flow tracer. Yet, experimental results for both impeller speeds display the contrary. Despite the behavior of the simulated alginate beads (hollow, dark yellow circles), for which the trend appears to change at v_{tip} , the linear trend for real alginate beads (filled, dark yellow circles) continues further to higher speeds, though with a comparatively low probability. In the study of Kuschel et al. a similar effect can be observed [16]. The harsh decline for the simulated alginate beads at the tip velocity speed may be explained by the effect of a smaller grid size than the particle diameter as mentioned previously. Furthermore, there is the possibility of a diameter overestimation of the experimentally manufactured alginate beads regarding the general calculation of the Sauter mean diameter $d_{32, \text{alg}}$ for an ellipsoidal object. Assuming the beads to be prolate ellipsoids, the surface area is calculated differently as $S_p = 2\pi w^2(1 + (q(w e)^{-1}) \cdot \sin^{-1}(e))$ (if $w < q$), whereas e stays the same as described in Appendix C. This would result in an alternative Sauter mean diameter of $d_{32, \text{alg, alt}} = 704 \cdot 10^{-6}$ m, and thus to an underestimation of the simulated velocity values, since smaller beads would move faster due to a decreased drag. However, numerical calculations assume whatsoever an ideally, spherically shaped alginate particle, which is, despite of a sphericity of

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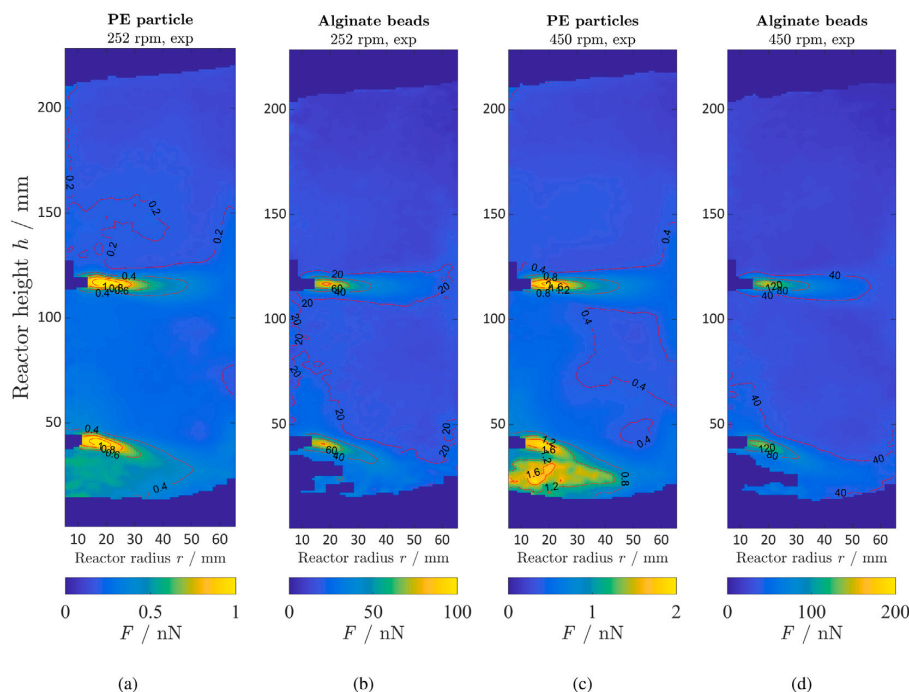


Fig. 9. Experimentally gained acting forces on (a, c) PE particles and (b, d) alginate beads for the impeller speeds of (a, b) 252 rpm and (c, d) 450 rpm calculated by means of Lagrangian acceleration magnitude data and corresponding particle masses for the front data slice (see Fig. 1). Data are time-, axially- and azimuthally-averaged over an axial and radial bin size of 1 mm. Data below $y = 30$ mm is neglected.

$\Psi = 0.998 \pm 0.003$ (with $\Psi = S_{\text{sphere}} S_p^{-1}$ and $S_{\text{sphere}} = \pi d_{32, \text{alg}}^2$) not the case for the experiments. Single-point velocity vector statistics along the Lagrangian trajectories can be observed in Appendix G.

Even more important to evaluate is the Lagrangian acceleration magnitude single-point statistic, which is seen in Fig. 7(b), also shown as a double-logarithmic PDF plot. Thereby, the acceleration of the simulated massless flow tracer is calculated from successive time steps and marks the boundary for occurring maximum accelerations in the system. Clearly, experimental data show a generally higher acceleration compared to the simulated analogue. Moreover, the trends of the experimental data show a peak at low acceleration magnitudes, whereas in the same region the simulation data depict nearly a linear trend in the double-logarithmic plot. Interestingly, the difference between experimental and numerical data regarding maximum acceleration magnitudes of the respective particle type seem to stay consistent with increasing impeller speed. The reason for the acceleration underestimation of the numerical data might be the negligence of two forces, namely the Saffman lift force and the two-way fluid-particle coupling as the negligence of forces could have direct influence on the acceleration. Considering these in the model for simulating alginate beads yielded unstable simulations due to very large or infinite velocities, and thus are not determined in this study. Again, corresponding velocity and acceleration information under $y = 30$ mm (see Fig. 6) were removed because of the previously mentioned high distortion in the reactor bottom region.

As not only single-point acceleration magnitudes, but also single-point acceleration vectors along the Lagrangian trajectories in Cartesian coordinates are gathered with 4D-PTV, Fig. 8 depicts exemplary two PDFs of the Lagrangian acceleration vector in x-direction for 252 rpm (left) and 450 rpm (right). Lagrangian single-point accelerations in a PDF as a normalized value with the root-mean-square (rms) acceleration a_{rms} of the respective simulated, ideal fluid flow tracer has

the potential to reveal inertial particle behavior. In this case, as Bec et al. [34] explicitly shows for numerical data, inertial particles distribute heterogeneously in a turbulent flow and concentrate in regions with a smaller pressure gradient. As a consequence, a major reduction of the rms acceleration will be observed in this graph type.

This is what can be seen in Fig. 8 (other Cartesian coordinates a_y and a_z , see Fig. 22 in Appendix G). Here, the simulated fluid flow tracer marks the outer boundary for occurring maximum normalized accelerations. Nevertheless, similar to Fig. 7(b), in which experimental data denote higher accelerations, also here throughout a lower reduction of the corresponding rms compared to simulated data can be seen. Despite of their size and density difference, this data may deduce an equivalent flow behavior for both experimental, inertial particle types because of their small difference in this plot.

These Lagrangian acceleration magnitude data gathered in experiments also offer another insight into a 3 L STR as to be seen in Fig. 9 as a 2D contour-isoline plot for occurring forces on the particles for both inertial particle types and impeller speeds. Due to shadowing effects in the back of the reactor, generated during the recording by the shaft and baffles, in this particular case only the data points from the front part of $\sigma = 120^\circ$ (see Fig. 1) are taken into account. Acceleration magnitude data are axially- and azimuthally-averaged over an axial and radial bin size of 1 mm and also time-averaged for the full recording time and eventually multiplied by the mass of the respective particle type. By doing so, a heterogeneous force distribution throughout the reactor is derived. For all cases, the highest forces are naturally found in the area near and around the impellers. The range of maximum forces on PE particles is around $1 \cdot 10^{-9}$ N for 252 rpm and $2 \cdot 10^{-9}$ N for 450 rpm. Alginate beads experience higher forces at maximum $60 \cdot 10^{-9}$ N and $120 \cdot 10^{-9}$ N, respectively. For alginate beads an approximate 60-fold higher force compared to PE particles is observed, if the values in Fig. 9(a) and (b) or (c) and (d) are contrasted. In all cases, the

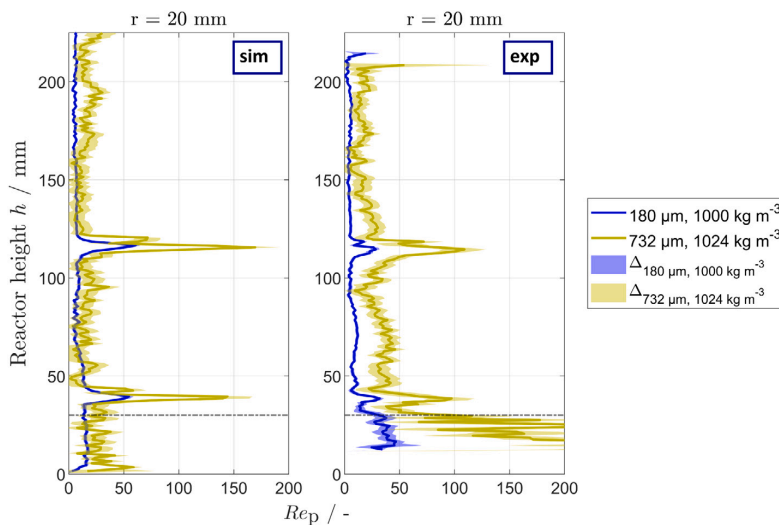


Fig. 10. Experimentally and numerically determined particle Reynolds number Re_p for the front slice (see Fig. 1) and 450 rpm over reactor height, for the radial position of 20 mm in the reactor, time-, axially- and azimuthally-averaged for a radial bin size of 4 mm and an axial bin size of 1 mm. Lines depict simulated (sim, left) and experimental (exp, right) data, respectively. Shaded areas show uncertainty Δ of respectively colored data and vertical bin.

prevailing force in the impeller region drops vastly with increasing distance towards the reactor bulk region. Numerical data is not shown here, however shows similar results. As mentioned previously, results under $y = 30$ mm are not discussed.

3.2. Evaluation of the particle Reynolds number

Certainly, the upper mentioned evaluations provide an overview regarding the Lagrangian velocity distributions, single-point statistics and averaged forces on particles. Yet, in order to represent the fluid turbulence and its associated particle flow-following behavior in a STR, a further discussion of the Stokes number of the mean flow St_{MF} (see Eq. (15)) is useful. To do so, a crucial intermediate step is to survey the particle Reynolds number Re_p (see Eq. (6)) in order to validate the boundary condition $1 \leq Re_p \leq 800$ for the consequent drag coefficient (see Eq. (8)) as a non-Stokesian correction factor.

Fig. 10 shows the Re_p for the most turbulent region in the reactor at $r = 20$ mm over the reactor height as calculated per Eq. (6). The radial bin size for calculating the Eulerian velocity average is chosen as 4 mm and the axial bin size as 1 mm. Most importantly, the averaged velocity has to be calculated for all three Cartesian grid coordinates separately, prior to calculation of the respective velocity average magnitude. As stated, the respective Eulerian undisturbed fluid flow information u is taken from the Lattice-Boltzmann simulations with massless tracer particles. Therefore, simulated (sim, left graph) and experimental (exp, right graph) data of both inertial particle types (PE particles: blue line, alginate beads: dark yellow line) are compared, considering that for each Re_p also the uncertainty Δ is calculated (respectively shaded area).

Basically, a general difference between the two particle types is evident for both simulations and experiments. The highest Re_p is observed in the direct impeller discharge regions, if values under $y = 30$ mm are neglected. No significant difference between simulation and experiment for the same particle type can be observed. The overall uncertainty values are plotted in similar magnitudes, respectively. Most importantly, Re_p never exceeds the critical value of 800.

3.3. Calculation of the Stokes number of the mean flow

The flow-following behavior of inertial particles in a fluid flow can be characterized by the prevailing Stokes number in the investigated

flow regime. Literature results suggest a particle to be a good flow tracer with $St \approx 10^{-2}$ for a sole consideration of single-point flow statistics [48]. A more general expression is found in Crowe et al. [46] as per $St \ll 1$ for a particle and the surrounding fluid to be in a nearly equilibrium. In this work the Stokes number of the mean flow St_{MF} [54], which considers the mean fluid flow (see Eq. (12)) in the respective bin is investigated. The characteristic length scale L is set as 1 mm and so is the size of the radial and axial bins to calculate the Stokes number as per Eq. (15).

Fig. 11 depicts the distribution of St_{MF} in a 3 L STR for both particle types and both chosen impeller speeds of experimental data. The particle Reynolds number is calculated for the identical grid by means of the averaged Lagrangian velocity for all three Cartesian grid coordinates and the averaged slip velocity with u from the respective simulation to gain the undisturbed fluid flow velocity. Similarly, only the data from the front slice of the reactor (see Fig. 1) are taken into account. The discharge flow region of both impellers towards the vessel wall and their direct vicinity show the highest Stokes number magnitudes, as approx. 0.4 and 0.6 for PE particles in the case of 252 rpm and 450 rpm, respectively.

Table 1

Overview of respective experimental and numerical average Stokes numbers $St_{MF,avg}$ and average particle Reynolds numbers $Re_{p,avg}$ for both particle types and impeller speeds. Data points under $y = 30$ mm neglected and only front slice considered (see Fig. 1).

Impeller speed	Particle and data type	$St_{MF,avg}$	$Re_{p,avg}$
252 rpm	PE, sim	0.1	2.8
	PE, exp	0.1	4.8
	alg, sim	1.1	15.0
	alg, exp	1.1	19.2
450 rpm	PE, sim	0.2	4.4
	PE, exp	0.1	8.1
	alg, sim	1.8	21.1
	alg, exp	1.6	34.6

Alginate beads illustrate a similar behavior, though, generally 10-fold higher Stokes numbers for both speeds. These plots represent clearly that a heterogeneous distribution of Stokes numbers exists throughout the reactor. The underlying velocity data and respective

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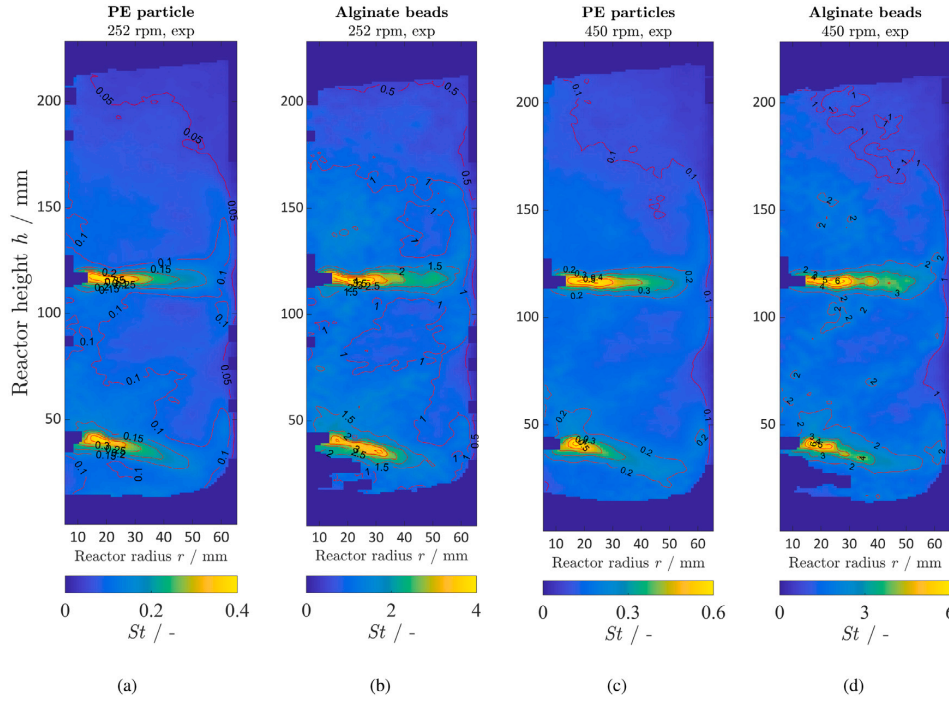


Fig. 11. Experimentally determined Stokes numbers of the mean flow St_{MF} (see Eq. (15)) for the front slice (see Fig. 1) for (a, c) PE particles and (b, d) alginate beads for the impeller speeds of (a, b) 252 rpm and (c, d) 450 rpm. Data are time-, axially- and azimuthally averaged over an axial and radial bin size of 1 mm.

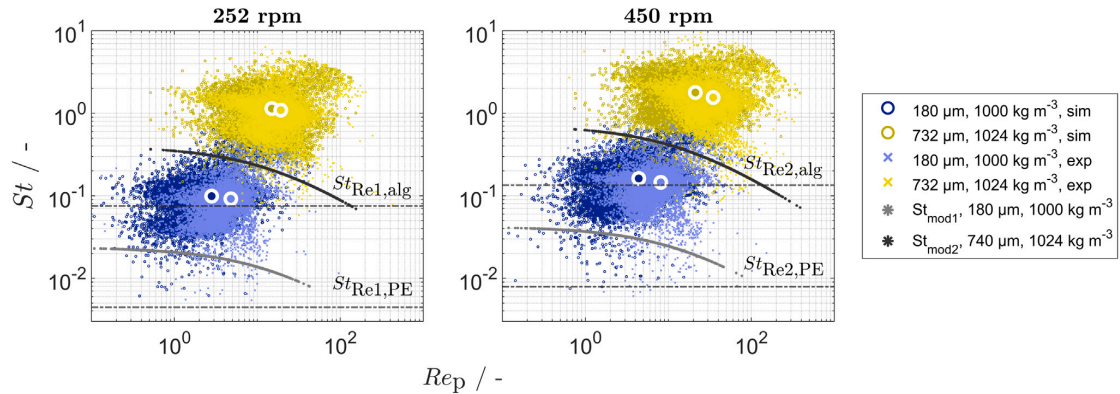


Fig. 12. Numerically (dark blue and dark yellow circles) and experimentally (light blue and yellow crosses) determined particle Reynolds numbers and Stokes numbers of the mean flow St_{MF} (see Eq. (15)) for the front slice (see Fig. 1), time-, axially- and azimuthally averaged over an axial and radial bin size of 1 mm. Bins under $y = 30$ mm are neglected. Respectively colored bigger circles denote the average value of the data set. For the purpose of comparison, differently calculated Stokes numbers are presented: dashed gray lines (St calculated by means of Eq. (14) with Re_{STR} instead of Re_t and $L_{STR} = 47$ mm) and light/dark gray data points st_i calculated by means of Eq. (13), connoted as St_{mod} .

uncertainties are shown in Figs. 23 and 24 in Appendix H. Interestingly, Fig. 13, in which solely numerical results on the same particles are evaluated, shows a similar performance regarding velocity vector differences in these critical areas around the impellers. The combination of both results show a qualitative examination through the reactor zones.

Moreover, a comparison between all numerically (dark blue and dark yellow circles) and experimentally (light blue and yellow crosses) calculated Stokes and particle Reynolds numbers in all 11 880 bins (bins under $y = 30$ mm are neglected) for each data set is sketched in Fig. 12. After all, the respective point clouds of each data set are in

a good agreement if simulations and experiments are compared with each other. An additional overview offers Table 1 with all average values. In the course of comparison of the particle Reynolds numbers, a shift towards higher values for experiments is prominent. This effect was already evident, when observing the velocity data in Fig. 7(a). However, the Stokes numbers for the respective impeller speed data sets align well, even though for the calculation of the experimental Stokes data and the corresponding slip velocity the undisturbed fluid flow velocity data u is taken from simulations.

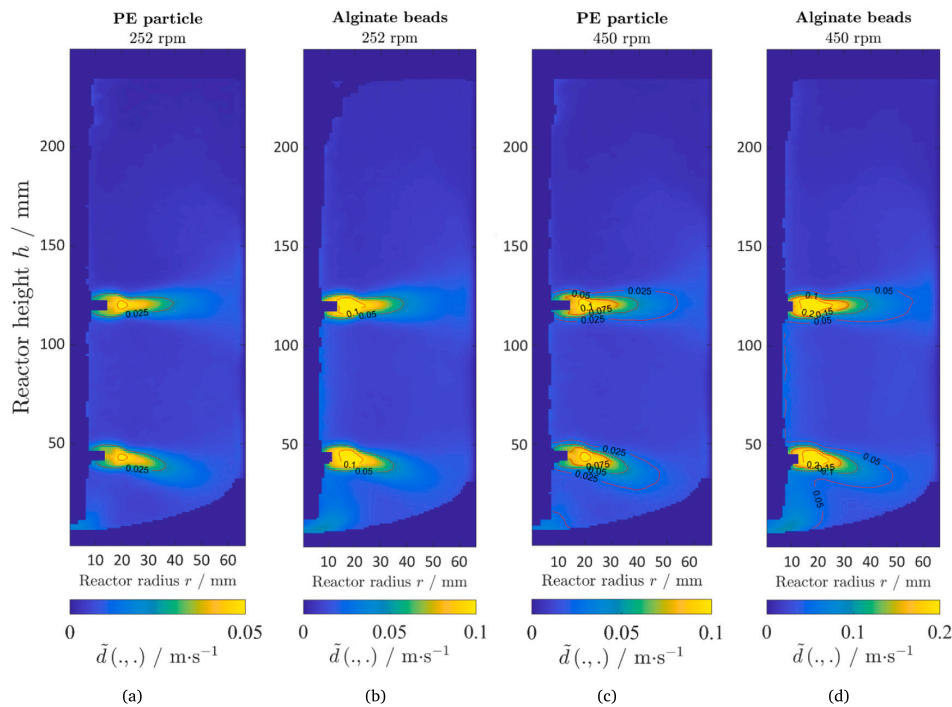


Fig. 13. Averaged Euclidean difference between the velocity of the fluid and the (a, c) PE particle or (b, d) alginate bead for both impeller speeds (a, b) 252 rpm and (c, d) 450 rpm.

In addition, Fig. 12 shows two horizontal lines and two curved trends of points in each plot. The first two (gray dashed lines) depict the theoretical Stokes number $St_{Re,alg}$ and $St_{Re,PE}$, if Eq. (14) and, on that account, the stirrer Reynolds number Re_{STR} (see Eq. (1)) instead Re_f would have been used for the overall calculation. In this case, the characteristic length scale L is chosen as $L_{STR} = 0.5(d_1 - d_2)$, which is the distance between the impeller tip and the vessel wall. The latter, marked as St_{mod} (light and dark gray dots) describes another variation of calculating the Stokes number, namely using Eq. (13) for the relaxation time of the fluid. Notwithstanding, both ways of estimating the Stokes number denote much smaller values compared to the method described in this study.

3.4. Distance between inertial and ideal particles and investigation of velocity vectors

In this subsection only numerical data is presented. To depict the simulated growing distance between the inertial and the ideal particles due to inertial effects which had their initial position at the very same point Fig. 14 shows the squared distance averaged over all particles for $0 \leq t \leq 0.1$ s. As can be seen, the mean squared distance grows for the first time steps as a quadratic function of time.

This behavior was yet stated by Ouellette et al. [48]. The averaged mean squared distance as function of times is retrievable by the application of the method of normal equations. The mathematical behavior in the quadratic regime can be found in Appendix J. Fig. 14 shows a higher displacement for the alginate beads for each stirrer frequency as expected from the higher Stokes numbers.

Furthermore, the difference between the velocity of the inertial particles and the fluid velocity was compared (Eq. (30)). This was done for every particle at every time step. The reactor was divided into bins separated by height and radius. Each bin was defined by a height of

$\Delta h_{bin} = 1$ mm and a radius of $\Delta r_{bin} = 1$ mm. Fig. 13 shows the averaged value for each of these bins over the whole range of the azimuthal $0 \leq \theta < 2\pi$ over the whole duration of the simulation.

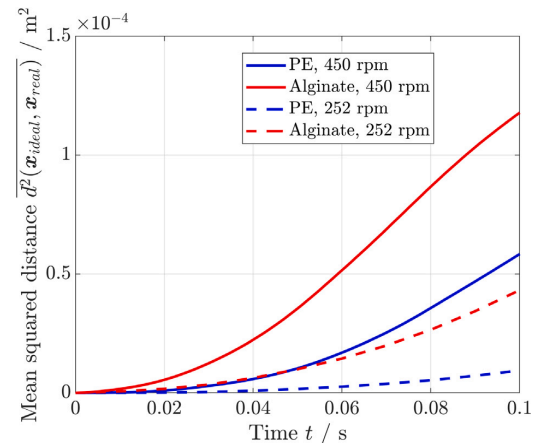


Fig. 14. Simulated mean squared Euclidean distance between the position of ideally flow-following and inertial particles over time, pairwise initialized at the same position and depicted for 252 rpm (dashed line) and 450 rpm (solid line).

Altogether, there is a visual similarity of this numerical data and the experimental data resulting in the Stokes number shown in Fig. 11. For both types of particles there is a relatively high difference to the velocity of the fluid in the region of the stirrers where large

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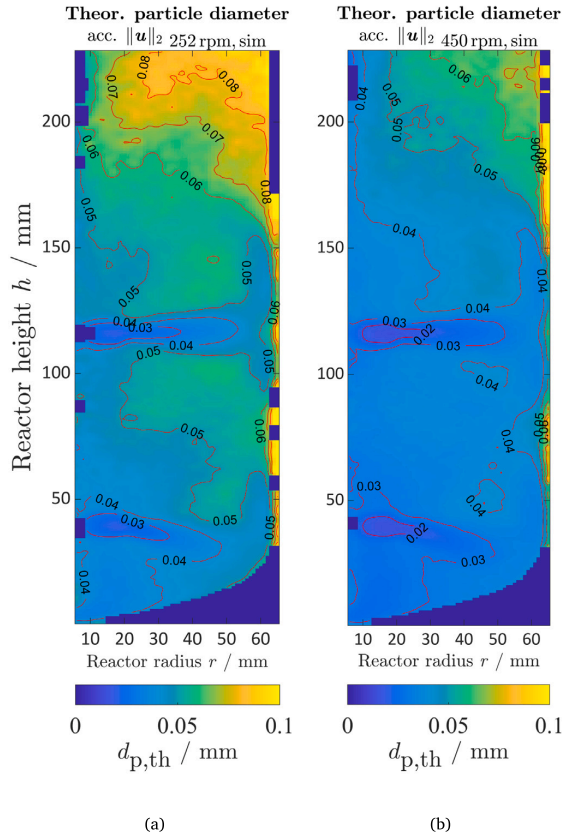


Fig. 15. Theoretical, maximum particle diameter $d_{p,th}$ distribution throughout the 3 L STR according Eq. (32) to fulfill the flow-following criteria of $St = 10^{-2}$. Data are taken from the front slice (see Fig. 1) and calculated by means of the simulated, ideal, massless tracer particles for both impeller speeds (a) 252 rpm and for (b) 450 rpm, time-, axially- and azimuthally-averaged over a radial and axial bin size of 1 mm.

experimental Stokes numbers were observed. As the simulations show, the higher Stokes numbers can be explained by a lower flow-following capability of the particles. Since the averaged data is depicted in Fig. 13 the lowest value is positive. Nevertheless, there are particles, which are moving in the opposite direction of the fluid. This is the case due to the existing momentum the inertial particles exhibit. The difference of the angle φ (data not shown) matches the data of Fig. 13 qualitatively and as that is in accordance to the statements concerning the flow-following behavior without any contradiction.

In order to refer this knowledge to the applicability of sensor particles in a STR, it is useful to estimate a particle diameter, for which the sensors could actually follow the flow. According to the presented results, the particle size must be much smaller than the used PE particles ($d_{PE} = 180 \mu\text{m}$) in this study. Therefore, the particle relaxation time τ_p from Eq. (7) can be simplified by complying with the prerequisite of $St \ll 1$ so that $24/(c_D Re_p) = 1$. Hence, the theoretical particle diameter is

$$d_p = \sqrt{\frac{18 St \eta L}{\rho_p \|u\|_2}}, \quad (32)$$

where St is the Stokes number with an estimated value of 10^{-2} , η is the dynamic viscosity with 1.0016 mPa·s, L is the characteristic length scale with 1 mm, ρ_p is the particle density with 1000 kg·m⁻³

and $\|u\|_2$ is the velocity magnitude of the simulated, ideal, massless tracer particles, time-, axially- and azimuthally-averaged for the respective bin to depict the fluid velocity. Fig. 15 shows the result of this appraisal for both impeller speeds 252 rpm and 450 rpm. According to this approximation, in the impeller discharge regions, theoretical, maximum particle diameters of 30 μm (252 rpm) or even 20 μm (450 rpm) would be necessary that a particle has the ability to follow the fluid flow. The resulting Stokes number of the mean flow St_{MF} distributions for mentioned theoretical, maximum particle sizes are depicted in 2D contour plots in annex 1. Furthermore, following the scale-down approach ($R_d = d_p/d_2 = 19.7 \cdot 10^{-3}$), which is mentioned in the introduction, this must accordingly change to $R_{d, new, 252 \text{ rpm}} = 0.83 \cdot 10^{-3}$ and $R_{d, new, 450 \text{ rpm}} = 0.56 \cdot 10^{-3}$ and concludes to a Lagrangian sensor particle size of approximately 0.6 mm (252 rpm) or even 0.4 mm (450 rpm) in diameter for a production scale reactor with a diameter of 2000 mm and an impeller diameter d_2 of 660 mm. In order to cover at least the flow-following behavior in the reactor bulk region, approximate sensor sizes of 0.9 mm or 0.7 mm would be necessary, respectively. This is not yet possible from the technical point of view and illustrates once again how important it is to further validate inertial particle simulations by experimental results on different scales in the short term and to invest in research on flow-following miniaturized, Lagrangian sensor particles in the long term.

4. Conclusions

In this work, experimentally and numerically derived particle trajectory information with a concurrent estimation of the flow-following behavior of inertial particles in a fluid flow within a 3 L lab scale STR are analyzed. By means of 4D-PTV measurements and Lattice-Boltzmann simulations spatial and temporal highly resolved data was generated and compared to each other. Neutrally buoyant PE particles and heavy alginate beads are used to scrutinize their behavior and flow-following capacity regarding their velocity, acceleration and secondary characteristics as the particle Reynolds number and the Stokes number at two impeller speeds. This study offers a deep insight into the hydrodynamics of such bioreactors with downsized instrumented Lagrangian particle sensors and their flow behavior. These are especially promising when the processes involve certain microorganisms, like CHO (Chinese Hamster Ovary) cells, being prone to high shear forces during a cultivation or, regarding a scale-up to production volumes.

As a result, (i) experimentally derived Lagrangian accelerations and consequential forces on particles as a function of the position in the reactor are mapped. Highest acceleration zones are shown in the vicinity of both Rushton Turbines. In these regions alginate beads experience approximately 100-fold higher forces than PE particles according to Newton's second law. Hereinafter, (ii) vertical velocity profiles and single-point statistics show a good agreement between experimental and numerical results. Normalizing all acceleration data by the rms of the simulated fluid flow shows generally less deviation for experimental data compared to simulations. However, this statistical data is not sufficient to obtain information about the inertial particle's flow-following behavior. Subsequently, (iii) experimentally derived particle Reynolds numbers Re_p are shown for high-turbulent zones in order to validate the upcoming calculation of the Stokes number. Hereby, the particle Reynolds number does not exceed 800, which points out the further usage of the mentioned drag coefficient c_D in the materials and method section. Moreover, the (iv) Stokes number is depicted in another 2D contour plot as a function of the position in the reactor. Similarly to the force contour plot, the zones around the impellers show the highest Stokes number, this time approximately 10-fold higher for the alginate beads compared to the PE particles for both impeller speeds. When comparing experimental and numerical results, a rather good agreement of the average Stokes numbers St_{avg} are found. However, the particle Reynolds number differs in its value, which might be due to the previously mentioned aspects regarding the simulations.

Eventually, (v) the distance between each inertial particle type and ideal particles over time and the deviation of the particle velocity from the velocity of the fluid from simulations confirm the areas of high Stokes numbers as areas of poor flow-following behavior. The results of the simulations denote an approximately 2-fold higher Euclidean velocity vector difference between the alginate beads and the fluid as the PE particles exhibit in the vicinity of the impellers.

In summary, the 4D-PTV method in combination with Lattice-Boltzmann simulations offer a good way to achieve deeper understanding about hydrodynamics of inertial particles and about the applicability of Lagrangian sensor particles in an STR. According to the Stokes numbers and the velocity difference between the particles and the fluid, the particles show a low flow-following capacity in almost the complete vessel, bearing the literature statements in mind. Especially in the vicinity of the stirrer, the investigated particles show a very poor flow-following behavior and actual instrumented sensor particles are likely to show the same behavior at bigger scales.

That is the reason why, based on these results, a theoretical, spatially resolved, maximum particle size is calculated in order to demonstrate the magnitude of Lagrangian sensor particle sizes necessary to fulfill the requirement to be flow-following. The results show all over the entire reactor domain lower values than the particle sizes used in this study. As industrial sensor particles are likely to behave in the same manner their capability to follow the flow is not necessarily given. According to the scale-down approach of particle-reactor diameter ratio, necessary sizes of smaller than 1 mm in diameter are estimated that an inertial sensor particle is able to follow the flow in the bulk region inside an industrial scale reactor.

Further analysis is to be conducted to determine the formation of compartments and to identify areas of different mixing behavior to clarify whether these particles, and thus sensor particles are able to cover the entire reactor domain. Therefore, it is necessary to study the difference of sensor particle trajectories and cell lifelines to ascribe the sensor data to spatial and temporal cell conditions during a cultivation process.

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.

Data and code availability

Exemplary segments of raw data and used MATLAB® scripts can be accessed at our DaRUS repository (doi: <https://doi.org/10.18419/darus-2294>).

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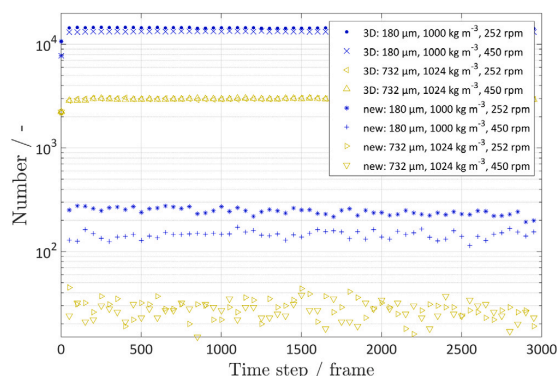


Fig. 16. Logarithmically plotted number of active tracks (3D) and concurrent added new tracks (new) while processing in the DaVis software for all 3000 time steps.

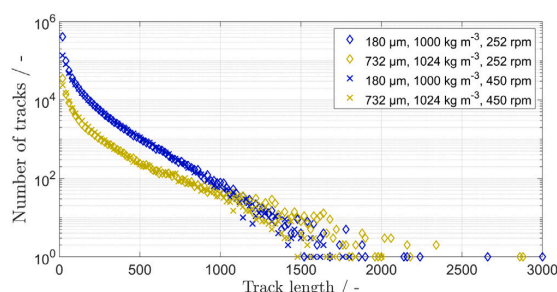


Fig. 17. Probability density function of the distribution of track number with its corresponding track length, separated in 150 bins.

Appendix A. Track length statistics

Fig. 16 shows the number of active tracks (3D) and simultaneously added new tracks (new) in the current time step for both types of particles (PE particles and alginate beads) at two different impeller speeds of 252 and 450 min⁻¹.

The minimum track length required was set to five consecutive tracks and velocity limits of $(0.0 \pm 1.0) \text{ m s}^{-1}$ and $(0.0 \pm 1.7) \text{ m s}^{-1}$ for the respective impeller speeds cover the maximum particle speed. Furthermore, an allowed triangulation error of 2.5 voxel for all analyses and acceleration limits with a maximum absolute change in particle shift of 4.5 and 6.5 voxel and a maximum relative change in particle shift between 30% and 35% were set, respectively. Higher acceleration limits led to more artifacts and falsely tracked particles in the reactor bottom and in shadowed areas as behind the shaft and in the baffle regions. As Schanz et al. [44] described, the first data point of each set in the graph depicts the convergence phase, in which all new tracks are added and upcoming particle positions need to be predicted and refined. All other data points represent the converged phase, where the number of tracks do not change in a significant manner. Theoretically, new tracks are only added, once particles enter the interrogation volume. In our particular case, the interrogation volume is a confined space and new particles are added once they appear again from behind the shaft, baffle or the reactor bottom, in which the refraction is still too high to triangulate within the allowed error.

The average number of active tracks for the PE particles (blue data points) is at $1.44 \cdot 10^4$ and $1.33 \cdot 10^4$ for 252 rpm and 450 rpm respectively, whereas the alginate beads (dark yellow data points) count on average $3.0 \cdot 10^3$ for both impeller speeds, which is due to the difference of the

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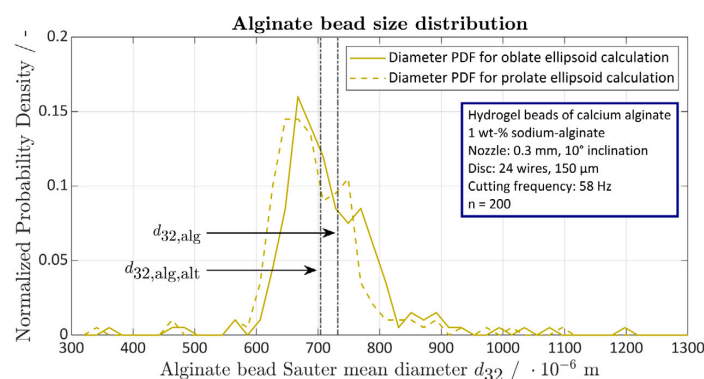


Fig. 18. Normalized PDF of the measured Sauter mean diameter $d_{32,alg}$ and $d_{32,alg,alt}$ of the fluorescent alginate beads ($n = 200$) due to two calculation types based on an oblate and prolate ellipsoidal shape, respectively. The taken values are indicated as $d_{32,alg} = 732 \cdot 10^{-6}$ m and $d_{32,alg,alt} = 704 \cdot 10^{-6}$ m.

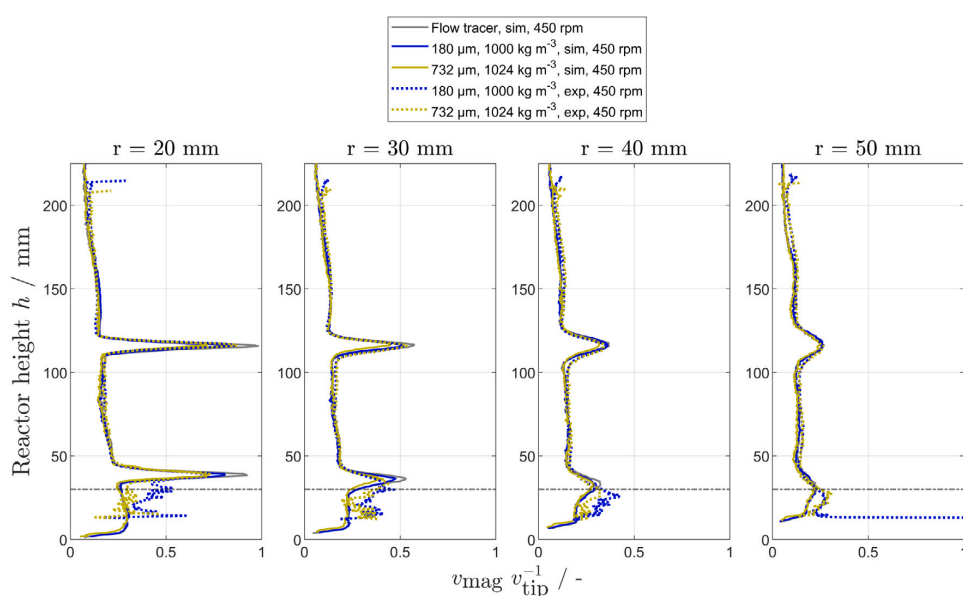


Fig. 19. Normalized velocity magnitude profiles over the reactor height (for 450 rpm) for four radial positions in the reactor, time-, axially- and azimuthally-averaged for a radial bin size of 4 mm and an axial bin size of 0.5 mm. Simulated (solid lines) and experimental results (dashed lines) are compared qualitatively. Gray dashed line at $y = 30$ mm depicts a cut-off for upcoming statistical evaluation due to high distortions in the reactor bottom.

total number of used particles at approx. 17 000 and 3800. We see a higher average rate of added tracks for the PE particles at 250 and 150 tracks per time step compared to the alginate beads at 30 and 28 per time step.

More information about the track length is gained from Fig. 17, which shows the number of active tracks and the corresponding track length in a probability density function plot. The difference in seeding density is also clearly seen between both the PE particles and alginate beads, resulting in an average track length of 58 and 89 (for PE particles) and 98 and 105 (for alginate beads). Due to the strongly skewed trend towards longer tracks it is necessary to mention the median values, which are at 16 and 41 (for PE particles) and 32 and 46 (for alginate beads), respectively.

Appendix B. Alginate bead preparation

By means of the jet cutting method (Jet Cutter: geniaLab Biotechnology GmbH, type S: S1702 A), demonstrated by Prüße et al. [70,71], Preibisch et al. [72] and Schroeter et al. [73], a 1 wt-% sodium-alginate (Hydagen 500, BASF) solution including 5 g l⁻¹ of orange fluorescent powder (Kremer Pigmente GmbH & Co. KG, Aichstetten, Germany) is used to form hydrogel beads in a container filled with 5 g l⁻¹ calcium chloride dihydrate (Carl Roth) solution. For this purpose, the liquid alginate solution is first pumped with a mass flow of 24.6 g min⁻¹ (corresponding to a jet velocity of 5800 mm s⁻¹) through a nozzle with a diameter of 0.3 mm, which has an inclination angle of 10°. Subsequently, the thin jet is cut by a disc with 24 wires of 150 μm diameter each, using a cutting frequency of $n_2 = 58$ s⁻¹ and a falling distance of 1.1 m. Due to the ionotropic gelation of the sodium alginate-solution with the calcium ions, eventually spherically shaped

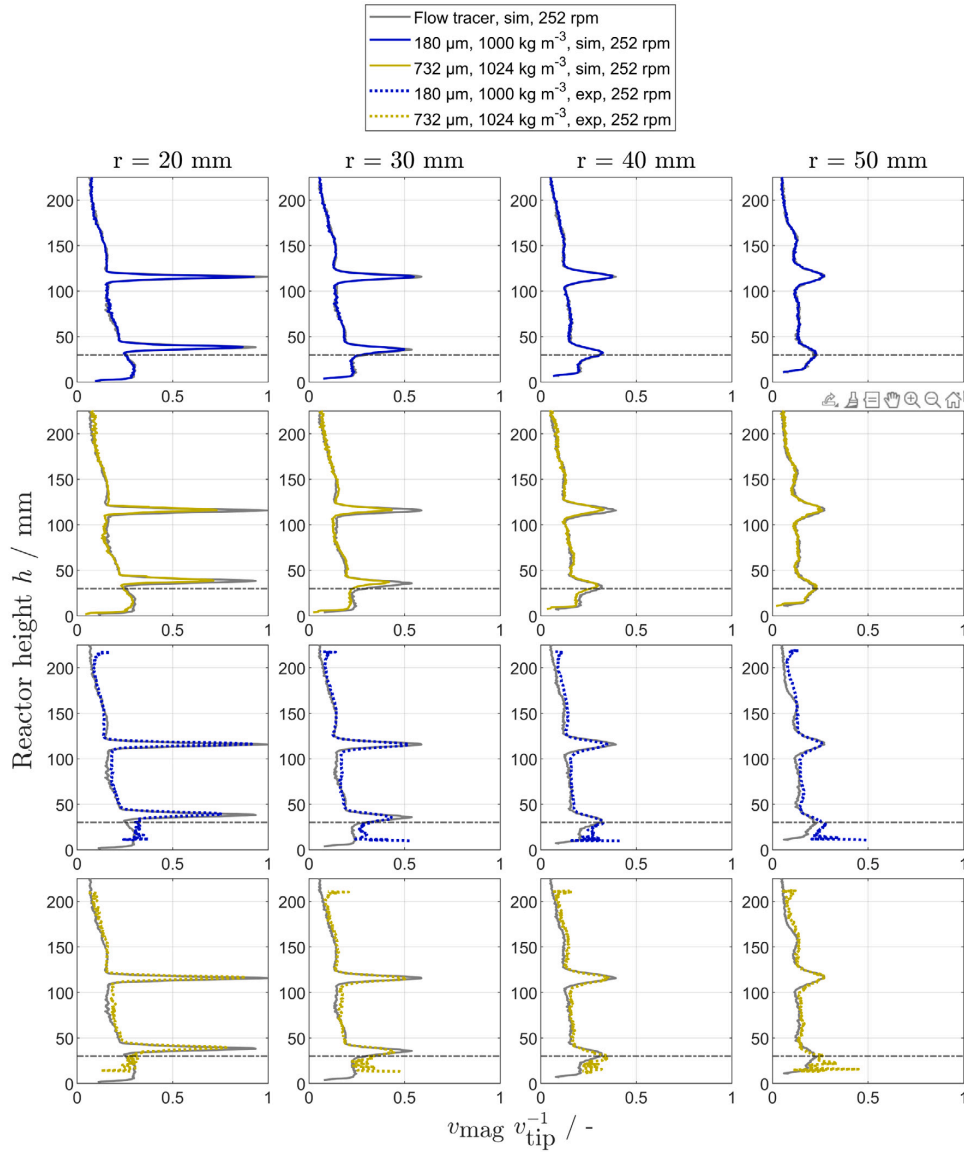


Fig. 20. Single plots for normalized velocity magnitude profiles over the reactor height (for 252 rpm) for four radial positions in the reactor, time-, axially- and azimuthally-averaged for a radial bin size of 4 mm and an axial bin size of 0.5 mm for simulated (solid lines) and experimental results (dashed lines). Gray dashed line at $y = 30$ mm depicts a cut-off for statistical evaluation due to high distortions in the reactor bottom.

calcium-alginate beads are formed. In this study, no further processing step is needed to use the particles as inertial tracers.

Appendix C. Alginate bead sphericity and Sauter mean diameter distribution

The bead sphericity Ψ_{alg} describes how close the shape of the particle resembles a perfect sphere. Hence, if the shape of an alginate bead is assumed as an oblate spheroid, the following Eq. (33) is used

to express the bead sphericity value:

$$\Psi_{\text{alg}} = \frac{2\sqrt[3]{qw^2}}{q + \frac{w^2}{\sqrt{q^2-w^2}} \ln \left(\frac{q+\sqrt{q^2-w^2}}{w} \right)}, \quad (33)$$

in which q and w are the semi-major and semi-minor axes, respectively. The Sauter mean diameter is calculated according to $d_{32, \text{alg}} = 6 \sum_{n=1}^{200} V_{p,i} (\sum_{n=1}^{200} S_{p,i})^{-1}$, whereas $V_p = 4/3\pi q^2 w$ is the particle volume and S_p its surface area. It follows $S_p = 2\pi q^2 (1 + ((1-e^2)e^{-1}) \cdot \tanh^{-1}(e))$ (if $w < q$), where e is the eccentricity with $e = \sqrt{1 - (w/q)^2}$. The normalized probability density plot of the alginate bead Sauter mean diameter can be seen in Fig. 18.

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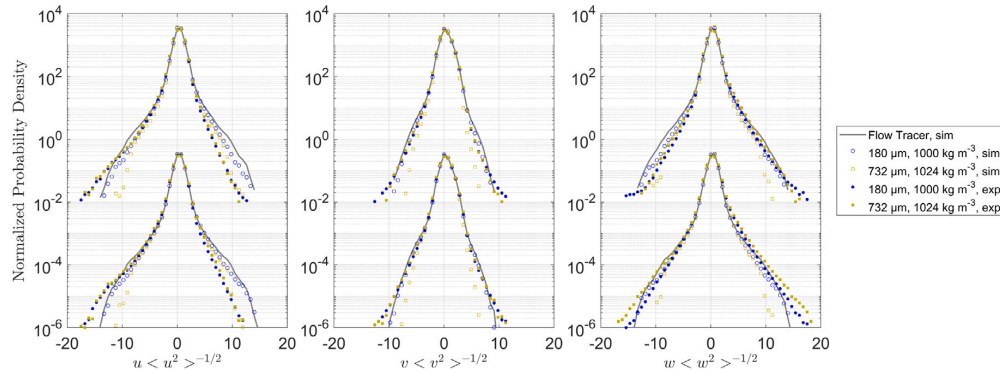


Fig. 21. Normalized PDFs of the Lagrangian particle velocities u (x -direction, width), v (y -direction, height) and w (z -direction, depth). The PDFs for different impeller speeds are offset for clarity. From bottom to top: 252 rpm and 450 rpm. The velocities are normalized by the rms value taken from the simulated, ideal fluid flow tracers (gray line). All data under $y = 30$ mm is removed due to high distortions in the reactor bottom.

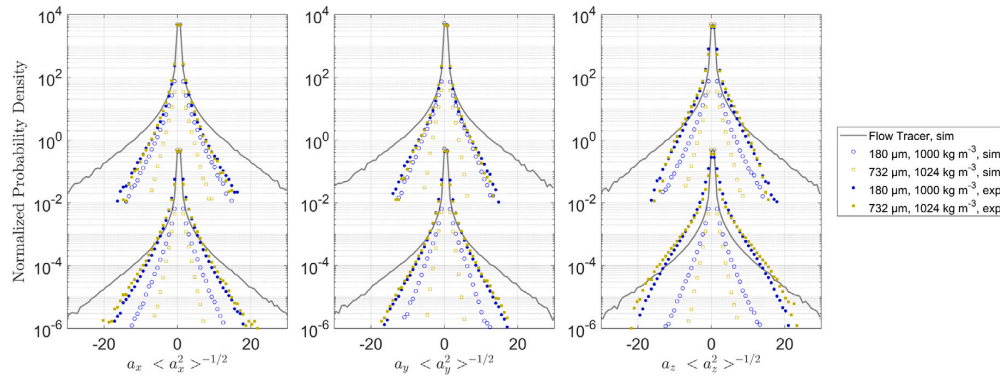


Fig. 22. Normalized PDFs of the Lagrangian particle accelerations a_x , a_y and a_z . The PDFs for different impeller speeds are offset for clarity. From bottom to top: 252 rpm and 450 rpm. The accelerations are normalized by the rms value taken from the simulated, ideal fluid flow tracers (gray line). All data under $y = 30$ mm is removed due to high distortions in the reactor bottom.

Appendix D. Calculation of Kolmogorov length scale, mean energy dissipation rate and discussion of drag coefficient correction factor

It needs to be mentioned that the influence of the free stream turbulence on the drag force becomes significant, if $d_p/\lambda > 5 - 10$ [74,75], whereas λ is the designated Kolmogorov length scale

$$\lambda = \left(\frac{\nu^3}{\epsilon} \right)^{1/4}. \quad (34)$$

Here, ν denotes the kinematic viscosity and $\bar{\epsilon}$ the mean energy dissipation rate as a good approximation for a STR [59,76]:

$$\bar{\epsilon} = \frac{P}{V \rho_f}, \text{ with} \quad (35)$$

$$P = Ne \rho_f n_2^3 d_2^5. \quad (36)$$

With n_2 as the impeller speed and the Newton number $Ne = 7.5$ for both Rushton turbines, the mean energy dissipation rate is calculated as $\bar{\epsilon}_{252 \text{ rpm}} = 1.2 \cdot 10^{-2} \text{ m}^2 \text{ s}^{-3}$ and $\bar{\epsilon}_{450 \text{ rpm}} = 6.8 \cdot 10^{-2} \text{ m}^2 \text{ s}^{-3}$. Furthermore, this results in $\lambda_{252 \text{ rpm}} = 96 \cdot 10^{-6} \text{ m}$ and $\lambda_{450 \text{ rpm}} = 62 \cdot 10^{-6} \text{ m}$. Hence, the ratios of $d_p/\lambda_{252 \text{ rpm}}$ are 1.9 and 7.6, along with $d_p/\lambda_{450 \text{ rpm}}$ as 2.9 and 11.8 for the PE particles and alginate beads, respectively. Therefore, the interaction between the energy dissipating eddies and particles can be neglected for PE particles, which leads to the conclusion that the drag coefficient c_D is unaffected by turbulence [74].

However, especially in case of 450 rpm it is assumed that the alginate beads might interact with the energy dissipating eddies and the bead's surrounding fluid, theoretically leading to a higher drag coefficient. Interestingly, if taking ϵ_{loc} into consideration, which is the local specific energy dissipation rate in a STR the values are in good agreement with Werner [76], meaning $\epsilon_{loc}/\bar{\epsilon} = 30$ in the immediate proximity of the impeller tip. If using this value, Brucato et al. [74] propose a drag coefficient correction factor of

$$\frac{c_{D,corr} - c_D}{c_D} = 8.76 \cdot 10^{-4} \left(\frac{d_p}{\lambda} \right)^3, \quad (37)$$

which results in $(c_{D,corr} - c_D)/c_D$ of approx. 27.6 for the alginate beads. This would eventually further decrease St in the impeller discharge region. Another study considering turbulent flows by Torobin and Gauvin [77] showed an influence on the drag coefficient at higher Re_p numbers, than calculated in this study. Moreover, Cisse et al. showed for similar particle volume fractions Φ_V and Kolmogorov length scales of $\lambda = 115 \cdot 10^{-6} \text{ m}$ evidence that used finite-size, neutrally buoyant particles have no impact on small-scale turbulent properties in a turbulent von Kármán flow experiment [58]. In conclusion, we decided on using Eq. (8) for all experimental and simulated data bearing in mind that for 450 rpm the results need to be treated with some caution.

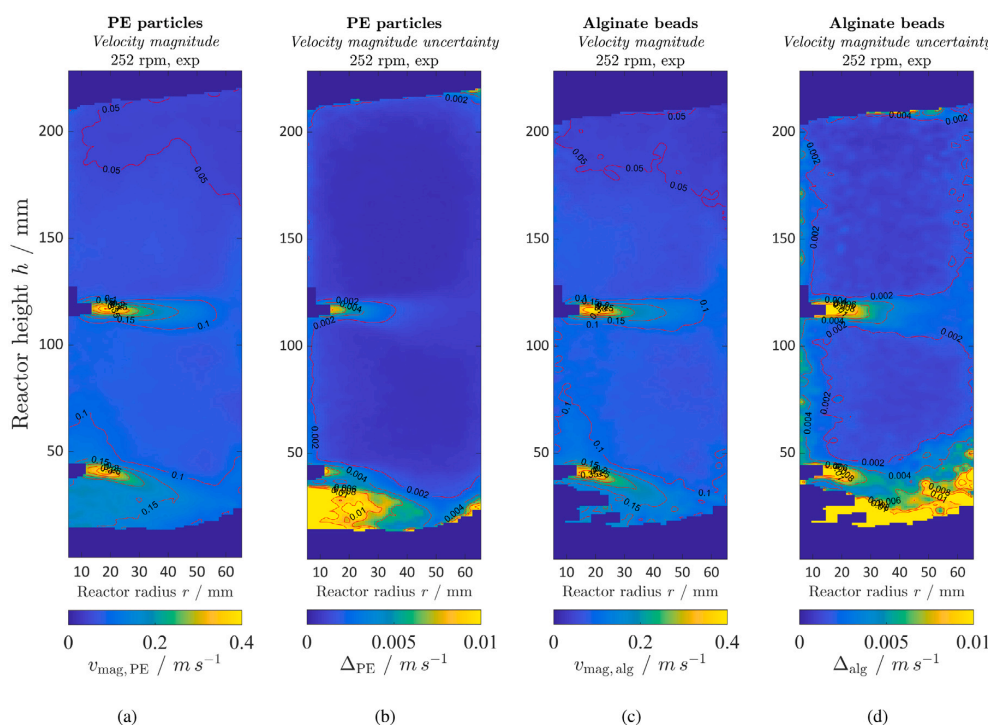


Fig. 23. Experimentally determined velocity magnitudes v_{mag} and corresponding uncertainty Δ for the front slice (see Fig. 1) for (a, b) PE particles and (c, d) alginate beads for the impeller speed of 252 rpm. Data are time-, axially- and azimuthally averaged over an axial and radial bin size of 1 mm.

Appendix E. Velocity magnitude profiles over the reactor height for 450 rpm

See Fig. 19.

Appendix F. Single velocity magnitude profiles over the reactor height for 252 rpm

See Fig. 20.

Appendix G. Statistical evaluation of single-point velocity and acceleration data in all Cartesian grid coordinates

Fig. 21 shows the three PDFs of the respective Lagrangian velocity vectors. The x and z direction represent horizontal movement, whereas the y direction stands for vertical movement in the reactor (see Fig. 1). For clarity, the two impeller speeds 252 rpm (lower graph) and 450 rpm (upper graph) are plotted on top of each other with an offset. Generally, all data points, numerical and experimental, are normalized by the rms value of the respective simulated, ideal fluid flow tracer (gray line) and plotted as a normalized PDF. This allows a statement regarding divergence of the particle's behavior compared to the surrounding fluid.

Our results show, as already similarly stated in [48], that there is no statistically significant difference in the main center regime between the single-point velocity for both impeller speeds investigated. Only a marginally skewed trend of u for both experimentally used particle types data can be observed towards the negative region.

Furthermore, Fig. 22 depicts the single-point acceleration statistics in all three Cartesian grid coordinates.

Appendix H. Calculation of the experimental velocity magnitude distribution and corresponding uncertainty throughout the 3 L reactor

Figs. 23 and 24 depict on the one hand the velocity magnitude v_{mag} distribution in form of a 2D contour plot and on the other hand the respective uncertainty for both PE particles (a, b) and alginate beads (c, d) at an impeller speed of 252 rpm and 450 rpm, respectively. In this case, only the Lagrangian data points in the front slice (see Fig. 1) of the reactor are taken into consideration, similarly to the evaluation of the Stokes number of the mean flow St_{MF} . Those data are time-, axially- and azimuthally averaged over an axial and radial bin size of 1 mm. Once again, it becomes evident that data points below the reactor height of 30 mm can be neglected for the evaluation of single point statistics or for the data shown in Fig. 12 due to a high uncertainty. Above that height, even the highest uncertainties in the impeller regions mark max. 5% and 3.5% of the velocity magnitude for 252 rpm and 450 rpm, respectively, which demonstrates the significance of the considered data.

Appendix I. Calculation of the Stokes number of the mean flow for maximum, theoretical particle sizes

According to Eq. (32), a theoretical, maximum particle size distribution throughout the reactor is calculated for both impeller speeds 252 rpm and 450 rpm and shown in Fig. 15. One assumption taken is the Stokes number value of 10^{-2} . Based on the results in that plot, the conclusion can be made that in the impeller discharge region particle sizes of approximately 30 μm and 20 μm , respectively, are necessary in order to follow the fluid flow, based on the assumption of the mentioned Stokes number value.

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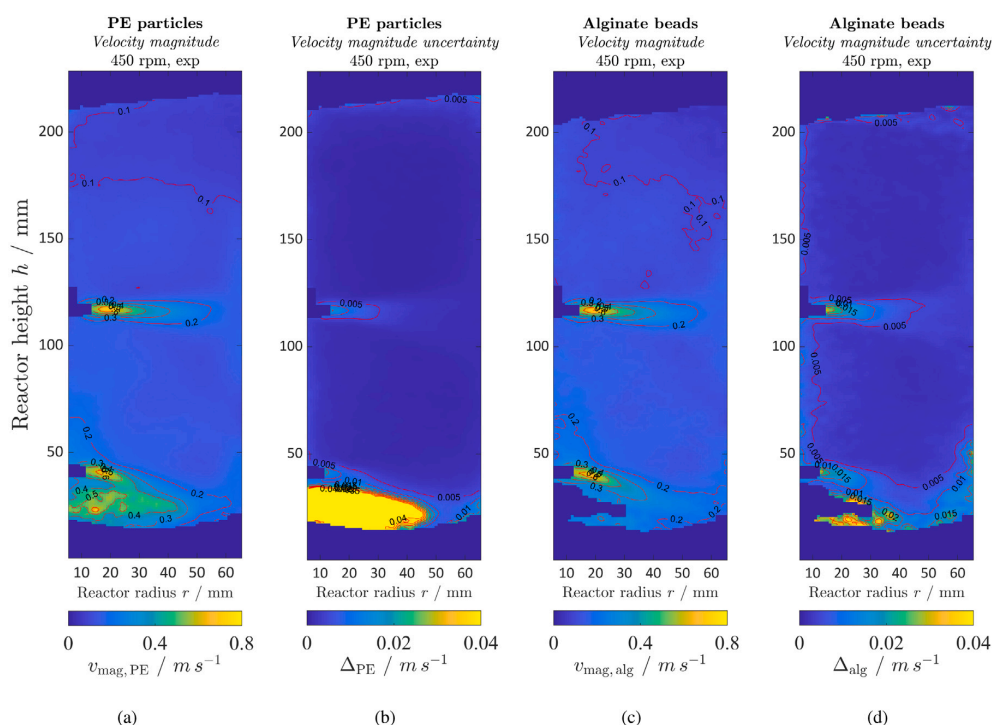


Fig. 24. Experimentally determined velocity magnitudes v_{mag} and corresponding uncertainty Δ for the front slice (see Fig. 1) for (a, b) PE particles and (c, d) alginate beads for the impeller speed of 450 rpm. Data are time-, axially- and azimuthally averaged over an axial and radial bin size of 1 mm.

On that account, two new sets of numerical simulations with mentioned particle sizes were conducted, the resulting Stokes numbers of the mean flow recalculated and depicted in a 2D contour plot for the respective impeller speed as to be seen in Fig. 25.

Appendix J. Averaged mean squared distance between inertial and non-inertial particles as function of time in the quadratic regime

PE particles, $n = 252$ rpm:

$$\overline{d^2(\mathbf{x}_{\text{ideal}}, \mathbf{x}_{\text{inertial}})} = 1.24 \cdot 10^{-3} \cdot t^2 - 3.27 \cdot 10^{-5} \cdot t + 1.79 \cdot 10^{-7}, \quad R^2 = 0.999$$

Alginate beads, $n = 252$ rpm:

$$\overline{d^2(\mathbf{x}_{\text{ideal}}, \mathbf{x}_{\text{inertial}})} = 4.70 \cdot 10^{-3} \cdot t^2 - 4.52 \cdot 10^{-5} \cdot t + 4.35 \cdot 10^{-7}, \quad R^2 = 1.000$$

PE particles, $n = 450$ rpm:

$$\overline{d^2(\mathbf{x}_{\text{ideal}}, \mathbf{x}_{\text{inertial}})} = 5.32 \cdot 10^{-3} \cdot t^2 - 7.02 \cdot 10^{-5} \cdot t + 2.59 \cdot 10^{-7}, \quad R^2 = 0.999$$

Alginate beads, $n = 450$ rpm:

$$\overline{d^2(\mathbf{x}_{\text{ideal}}, \mathbf{x}_{\text{inertial}})} = 1.51 \cdot 10^{-2} \cdot t^2 - 5.11 \cdot 10^{-5} \cdot t + 4.56 \cdot 10^{-7}, \quad R^2 = 1.000$$

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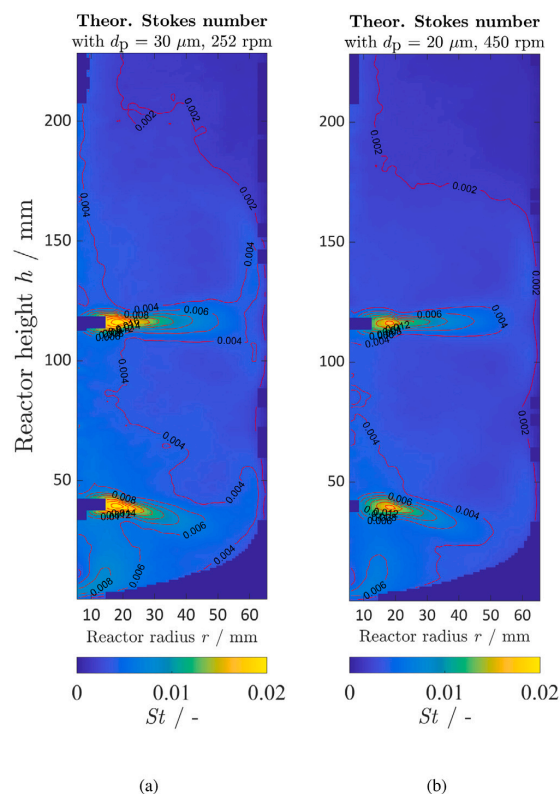


Fig. 25. Numerically determined Stokes numbers of the mean flow St_{MF} (see Eq. (15)) for the front slice (see Fig. 1) for the theoretical, maximum particle diameter $d_{p,th}$ as (a) 30 μm in the case for 252 rpm and (b) 20 μm in the case for 450 rpm (see Fig. 15). Data are time-, axially- and azimuthally averaged over an axial and radial bin size of 1 mm.

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Chapter 5

Experimental analysis of lifelines in a 15,000 L bioreactor by means of Lagrangian Sensor Particles

This chapter reproduces the publication: Hofmann, S., Buntkiel, L., Rautenbach, R., Gaugler, L., Ma, Y., Haase, I., Fitschen, J., Wucherpfennig, T., Reinecke, S., Hoffmann, M., Takors, R., Hampel, U., Schlüter, M., 2024. Experimental analysis of lifelines in a 15,000 L bioreactor by means of Lagrangian Sensor Particles. Chemical Engineering Research & Design 205, 695–712 [Hof24b]. Utilized data and mathematical analyses can be accessed at the DaRUS repository [Hof24a] or in the Gitlab repository [Hof25e].

Lifelines on industrial scale

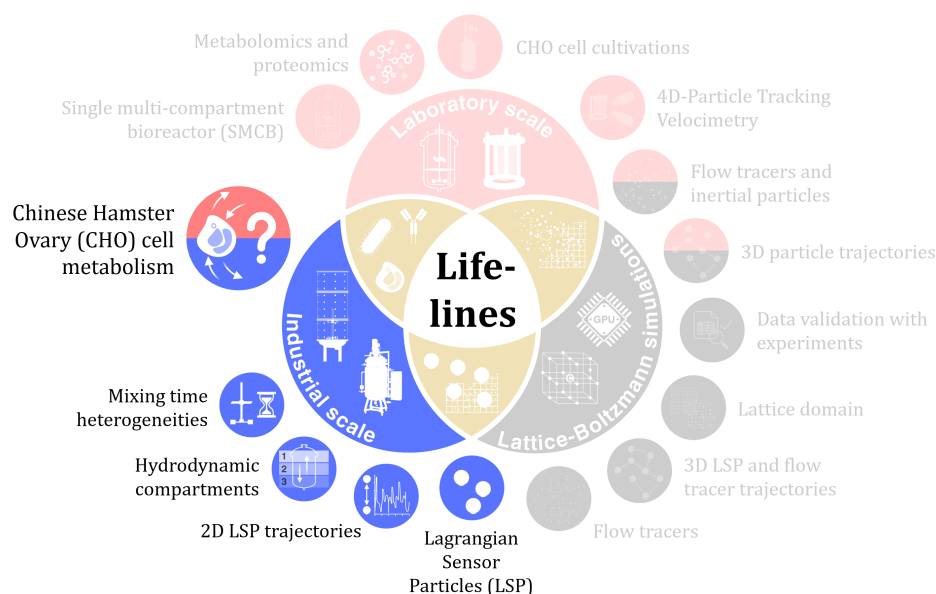


Figure 5.1: Figurative representation of the investigation framework of the second publication.

The ability to accurately reconstruct three-dimensional Lagrangian particle trajectories on laboratory scale, as demonstrated in the first publication, has significantly enhanced the physical understanding of inertial particles submersed in turbulent flows in a bioreactor. These detailed insights provide a solid foundation for interpreting particle dynamics and environmental conditions in industrial-scale applications. From the first publication, it becomes evident that current state-of-the-art Lagrangian Sensor Particles (LSPs) are unable to mimic cell lifelines due to their inertia, especially on micro-scale. A theoretical maximum particle diameter of $d_{p,th} \approx 1$ mm is estimated as the upper limit for flow-following behavior in a 15,000 L reactor in the bulk region. However, this first estimate is based on data obtained in a 3 L laboratory-scale reactor and needs to be treated with caution. Before addressing whether such micro-scale flow-following behavior is even necessary for industrial-scale characterization, it must first be determined what kind of information can generally be obtained with currently available LSPs with $d_{LSP} = 40$ mm and how these can benefit a fluid dynamic characterization. Can these LSPs provide meaningful hydrodynamic insights despite their limited flow-following capability? How do they perform on industrial scale, and can they resolve heterogeneities such as compartment boundaries and residence time distributions, which are critical parameters for designing scale-down models?

To address these questions, the second publication scrutinizes LSP trajectories on industrial scale, in a 15,000 L bioreactor equipped with three Elephant Ear impellers (Fig. 5.1).

The study evaluates the ability of LSPs to detect hydrodynamic compartments and mixing heterogeneities in a bioreactor setup, where originally optical access is not available, and where a "go-with-the-flow" approach [Dui20] offers a pragmatic strategy for exploring flow structures under real-world process conditions.

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Experimental analysis of lifelines in a 15,000 L bioreactor by means of Lagrangian Sensor Particles

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ABSTRACT

This study employs Lagrangian Sensor Particles (LSPs) with a diameter of 40 mm equipped with a pressure sensor to investigate cell lifelines in a 15,000 L stirred tank reactor (STR) with three Elephant Ear impellers. The Stokes number of the LSPs is approx. 0.004 on a macro-scale. The vertical probability of presence, axial velocity profiles, circulation time distributions, and residence time distributions are quantified to analyze single-phase mixing heterogeneities, detect hydrodynamic compartments and conduct a Lagrangian regime analysis. Results reveal a similarly distributed probability of presence in the vertical reactor center but emphasize the LSP's sensitivity to fluctuating densities. Axial velocity distributions illustrate characteristic impeller-induced flow patterns, and circulation time distributions identify three compartments with comparatively shorter times in the axial center. Residence time distributions exhibit a similar compartmentalized profile. Moreover, the study estimates a potential oxygen deprivation zone for CHO cells in the upper compartment and demonstrates the LSP's efficacy in characterizing impeller systems. Contrary to literature, the ratio of examined global mixing times to circulation times is 1.0, highlighting macro-scale mixing. The research underscores that LSPs offer crucial insights into industrial-scale STRs, specifically for determining hydrodynamic compartments without having optical access.

1. Introduction

In recent decades, the field of biotechnology has experienced substantial progress in genetic engineering, mammalian cell culture and genomics and proteomics, leading to the development of advanced biotechnological processes. For instance, after the development of artificial microbial consortia for biotechnological production processes on laboratory scale (Mittermeier et al., 2022; Herzog et al., 2023; Kratzl et al., 2023), large-scale bioreactors with volumes up to 30 m³, and more can be utilized (Larsson et al., 1996; Vrabel et al., 2000). To do so, process monitoring and process control are critical elements to ensure reproducibility, maintain high-quality standards and enhance efficiency and productivity. However, when scaling up bioprocesses, there is often a reduction in productivity (Nadal-Rey et al., 2021b). This may be attributed to the emergence of population heterogeneities within the culture (Nadal-Rey et al., 2021b; Hoang et al., 2022; Rugbjerg

and Sommer, 2019; Takors, 2012; Enfors et al., 2001; Heins et al., 2015), stemming from non-ideal dynamic conditions. Such temporal and spatial gradients originate from mixing heterogeneities within the bioreactor in form of compartments (Rosseburg et al., 2018; Kuschel and Takors, 2020; Nadal-Rey et al., 2021a; Weiland et al., 2023), which might furthermore emerge from an overestimated sensitivity of the cells to shear stress (Nienow et al., 2013), and therefore deteriorate key performance criteria such as titer, rates and yield (George et al., 1998; Lara et al., 2006). Similar scenarios and gradients can be investigated on microfluidic chips (Ho et al., 2019, 2022) or mimicked in scale-down reactors on laboratory scale by using e.g. flow-inhibiting elements or separate cultivation units. This enables to investigate cell-bioreactor interactions more thoroughly, as was reviewed by Neubauer and Junne (Neubauer and Junne, 2010), and more recently, by Nadal-Rey et al. (2021b). On that account, large-scale data regarding

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Nomenclature**Arabic symbols**

A	Area/m ²
Ar	Archimedes number/–
b	Baffle width/m
C	Impeller off-bottom clearance/m
c	Concentration/g L ⁻¹
c_D	Drag coefficient/–
d_{imp}	Impeller diameter/m
d_{LSP}	LSP diameter/mm
d_p	Particle diameter/mm
d_{STR}	Reactor diameter/m
f	Sampling frequency/Hz
g	Gravitational acceleration/m s ⁻²
H	Reactor filling height/m
h	Impeller spacing/m
K	Henry coefficient/Pa m ³ mol ⁻¹
k_{to}	Turn-over constant/–
L	Characteristic length scale/m
L_E	Taylor scale/m
M	Total number of counts in dataset/–
m_{LSP}	LSP mass/g
n	Impeller frequency/rpm
$N_{Q,c}$	Circulation Flow number/–
$N_{Q,e}$	Exchange Flow number/–
$N_{Q,p}$	Pumping number/–
$N_{Q,r}$	Residence Flow number/–
N_j	Number of counts in bin j /–
P	Power input/W
p	Pressure/Pa
$P V^{-1}$	Global power per volume input/W m ⁻³
p_0	Ambient pressure/Pa
P_o	Power number/–
r	Radial distance/m
Re_{STR}	Global Reynolds number/–
Re_p	Particle Reynolds number/–
St	Stokes number/–
T	Temperature/°C
t	Time/s
t_{circ}	Circulation time/s
u	Velocity of the fluid/m s ⁻¹
u_0	Root mean square of fluid velocity/m s ⁻¹
u_{tip}	Impeller tip speed/m s ⁻¹
V	Reactor working volume/m ³
v	Velocity of the LSP/m s ⁻¹
v_z	Axial velocity/m s ⁻¹
z	Specific height in reactor/m

Greek symbols

Δ	Uncertainty
δ	Difference
η	Dynamic viscosity/Pa s
λ_K	Kolmogorov length scale/m
$\lambda_{K,\Phi}$	Min. Kolmogorov length scale/m
ν	Kinematic viscosity/m ² s ⁻¹
Φ	Local energy dissipation ratio/–

ϕ	Sphericity/–
ρ	Density/kg m ⁻³
ρ_f	Fluid density/kg m ⁻³
ρ_p	LSP density/kg m ⁻³
τ	Residence time/s
τ_f	Characteristic time of the flow field/s
τ_p	Particle response time/s
ε_T	Energy dissipation rate/W kg ⁻¹
ξ^*	Mass concentration/kg m ⁻³

Abbreviations

CHO	Chinese hamster ovary
CTD	Circulation time distribution
DW	Deionized water
EE	Elephant Ear
HCl	Hydrochloric acid
HZDR	Helmholtz-Zentrum Dresden-Rossendorf
IP	Injection point
IS	Isotonic solution
LSP	Lagrangian Sensor Particle
NaCl	Sodium chloride
NaOH	Sodium hydroxide
PBT-4	4-bladed pitched blade turbine
PBT-6	6-bladed pitched blade turbine
rms	Root mean square
RT-6	6-bladed Rushton turbine
RTD	Residence time distribution
STR	Stirred tank reactor

circulation times (Reinecke et al., 2012), residence times (Siebler et al., 2019) and especially the locations of compartment barriers, volume flow exchange rates (Vrábel et al., 2000, 1999) and respective mixing times (Rosseburg et al., 2018) provide fundamental information to the implementation of a scale-down model (Gaugler et al., 2022; Noorman, 2011).

On industrial scale, aforementioned hydrodynamic process data can be typically obtained by means of two basic sensor measurement approaches: The Eulerian approach, which is the industrial standard, utilizes locally fixed probes, either at one specific position at the reactor wall or multiple probes distributed over the height or circumference of the reactor. The collected data delivers quasi-1-dimensional information about fermentation process conditions, such as temperature, pH, dissolved oxygen concentration or conductivity (Gargalo et al., 2020) over time. However, with these sensor types, neither the cell's duration of residence in individual metabolic regimes nor gradients can be resolved spatiotemporally. Only a coarse and locally constrained data density can be obtained, thus making it insufficient to deliver adequate values for scale-down reactor models. The Lagrangian approach is based on particles submersed in a liquid, which follow the fluid flow in a characteristic manner, depending on their physical properties. Through the implementation of multiple sensor systems within an inertial, finite-sized, mostly neutrally buoyant and freely moving Lagrangian Sensor Particle (LSP) (Fitschen et al., 2023; Buntkiel et al., 2023a), the primary objective is to understand cell-bioreactor interactions, while mimicking the movement of a cell. This 3-dimensional concept "Traveling along the Lifelines of Single Cells" in a stirred bioreactor was introduced by Lapin et al. (2004) and recently reviewed by Blöbaum et al. (2023) for both experimental and numerical methods. Regarding the latter, Haringa et al. (2016) have demonstrated an approach to gain statistical insights into metabolic regime analyses,

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eventually allowing for a quantification of substrate concentration variations with regard to scale-down models. This Lagrangian regime analysis, further investigated in following studies (Kuschel and Takors, 2020; Haringa et al., 2017, 2018), can depict frequency and magnitude statistics of environmental fluctuations in compartmentalized flows. However, experimental data is still required in order to further calibrate compartment models (Nadal-Rey et al., 2021a; Delafosse et al., 2014) and validate Lagrangian particle simulations (Hofmann et al., 2022; Kuschel et al., 2021).

Initial investigations regarding the utilization of active flow-followers for analyzing circulation times and their correlation to mixing times in a STR were conducted by Bryant and Sadeghzadeh (1979). Day (1975) discussed a log-normal distribution of measured circulation times with the same flow-followers. In addition, model parameters like the non-dimensional flow number and mixing times were validated. Their work is based on the development of “radio pills” by Mitchell (1969). Moreover, Middleton (1979) and Van Barneveld et al. (1987a,b) continued circulation time measurements in large-scale STRs of up to 20 m³, whereas Mann et al. (1981) focused on a stochastic flow model utilizing the respective data and emphasizing the careful interpretation of circulation time distributions (CTD) with regards to their length of experiments. In the past decades, a large number of research groups have continued investigating the application of LSPs in the context of biotechnology (Reinecke et al., 2012; Bisgaard et al., 2021b,a, 2020; Buntkiel et al., 2023b; Lauterbach et al., 2019; Reinecke et al., 2022; Stine et al., 2020b,a; Todtenberg et al., 2015), however, their application in industrial-scale STRs is rarely published.

Whereas Reinecke et al. used a single-staged pitched blade turbine (PBT) in STRs with volumes between 0.8 and 1.4 m³ (Reinecke et al., 2012; Reinecke, 2013), Bisgaard et al. (2021a) applied their LSPs in STRs with approx. 0.6 m³ and studied both a single-stage PBT and a single-stage Rushton turbine (RT) setup. Apart from these well-known impeller configurations, particularly for mammalian cell cultivations (Sieck et al., 2013), plant cell suspension cultures (O'Neill et al., 2008) or β -cell spheroid productions (Petty and Salzig, 2022), Elephant Ear (EE) impellers are widely utilized in STRs due to their enhanced bulk mixing characteristic attributed to a wide blade shape and a high impeller-to-reactor diameter ratio ($d_{\text{imp}} d_{\text{STR}}^{-1} \approx 0.5$). Nevertheless, in literature, this 3-bladed, 45° pitched impeller with a predominant axial flow direction has not been reported as of 2006 (Nienow, 2006) and since then mainly in two variations, named as the Applikon design (Collignon et al., 2010a,b; Delbridge et al., 2023; Rotondi et al., 2021; Simmons et al., 2007; Zhu et al., 2009) and the ABEC design (O'Neill et al., 2008; Petty and Salzig, 2022; Fitschen, 2021; Meier, 2005). Nonetheless, no mixing study, which considers a three-staged EE impeller ABEC design on industrial scale in a baffled STR has been published to date.

In that context, this study focuses on aforementioned issues and combines (i) the application of LSPs in a baffled 15,000 L STR, which is (ii) equipped with three EE impellers (ABEC design) in order to (iii) gather experimental data regarding circulation times and residence times, based on the calculation of the vertical probability of presence and axial velocities to localize compartments, compare these results with global mixing times for the same setup and conduct a Lagrangian single-phase hydrodynamic regime analysis. Moreover, dimensionless numbers as the Stokes number and different Flow numbers are estimated and compared to literature findings.

2. Materials and methods

In this section, the reactor setup and Lagrangian Sensor Particles (LSP) are described in detail. The description of pressure data evaluation is provided, meaning the calculation of the vertical probability of presence, determining the circulation time distribution, and calculating the hydraulic residence time distribution. Moreover, the experimental method for measuring the global mixing times in the aforementioned

Table 1

Overview of system parameters in deionized water and isotonic solution.

	Deionized water (DW)	Isotonic solution (IS)
Temperature $T/^{\circ}\text{C}$	(22 ± 0.3)	(37.0 ± 0.3)
Dynamic viscosity $\eta/\text{Pa s}$ ($\cdot 10^{-3}$)	0.95	0.71
Fluid density $\rho_f/\text{kg m}^{-3}$	(997.8 ± 0.4)	(1007.7 ± 0.4)
LSP density $\rho_p/\text{kg m}^{-3}$	$\rho_f \leq \rho_p \leq (\rho_f + 2)$	$(\rho_f - 1) \leq \rho_p \leq (\rho_f + 2)$
Archimedes number Ar ($\cdot 10^3$)/–	≤ 1.38	≤ 1.78

reactor and the approaches for estimating the Stokes number and different Flow numbers are described.

2.1. 15,000 L acrylic glass bioreactor and Elephant Ear impellers

For this study, a transparent acrylic glass bioreactor with an inner diameter of $d_{\text{STR}} = 2.0\text{ m}$, a filling level of $H = 4.2\text{ m}$ and a klöpper head is characterized hydrodynamically (Fig. 1). The reactor has been erected in cooperation with Boehringer Ingelheim Pharma GmbH & Co. KG (Rosseburg et al., 2018). The total working volume is $V = 12500\text{ L}$ of either deionized water (DW) at a temperature of $T = (22 \pm 0.3)^{\circ}\text{C}$ or an isotonic solution (IS) with sodium chloride ($c_{\text{NaCl}} = 9\text{ g L}^{-1}$) at a temperature of $T = (37 \pm 0.3)^{\circ}\text{C}$. The fluid densities are measured with $\rho_f = (998.0 \pm 0.4)\text{ kg m}^{-3}$ and $\rho_f = (1007.7 \pm 0.4)\text{ kg m}^{-3}$ (Anton Paar DMA 4500 M) and the corresponding dynamic viscosities are estimated with $\eta = 0.95 \cdot 10^{-3}\text{ Pa s}$ and $\eta = 0.71 \cdot 10^{-3}\text{ Pa s}$, respectively. The reactor is equipped with four equally spaced baffles of width $b = 0.1 \cdot d_{\text{STR}}$ and a bottom-driven magnetic agitator (ZETA BMRX XXL) with three Elephant Ear (EE) impellers having a diameter $d_{\text{imp}} = 0.915\text{ m}$, which corresponds to a ratio of $d_{\text{imp}} d_{\text{STR}}^{-1} = 0.46$. The off-bottom clearance $C = 0.3 \cdot d_{\text{STR}}$ and an impeller spacing of $h d_{\text{imp}}^{-1} = 1.0$. Applied stirring frequencies range from $n = 15\text{ rpm}$ (corresponds to $u_{\text{tip}} = 0.72\text{ m s}^{-1}$) to $n = 40\text{ rpm}$ (corresponds to $u_{\text{tip}} = 1.9\text{ m s}^{-1}$) (see Table 2).

With an estimated Power number of $Po = 5.3$ for this setup, the global volumetric power inputs

$$\frac{P}{V} = \frac{Po \cdot \rho_f \cdot n^3 \cdot d_{\text{imp}}^5}{V} \quad (1)$$

and stirrer Reynolds numbers

$$Re_{\text{STR}} = \frac{n \cdot d_{\text{imp}}^2 \cdot \rho_f}{\eta} \quad (2)$$

are mentioned in Table 2 for each respective medium. Furthermore, with those values, the mean specific energy dissipation rate $\bar{\epsilon}_T$ and the Kolmogorov length scale λ_K can be derived according to

$$\bar{\epsilon}_T = \frac{P}{V \rho_f} \quad (3)$$

and

$$\lambda_K = \left(\frac{\nu^3}{\bar{\epsilon}_T} \right)^{\frac{1}{4}}, \quad (4)$$

where ν denotes the kinematic viscosity of the fluid. Since the energy dissipation rate varies throughout the STR and is highest in the discharge flow of the impellers, a ratio of $\Phi = (\epsilon_T)_{\text{max}} (\bar{\epsilon}_T)^{-1} = 115$ is estimated for the respective region (Stahl Wernersson and Trägårdh, 1999) according to

$$\log(\Phi) = -1.04 \cdot \frac{2r}{d_{\text{imp}}} + 3.1, \quad (5)$$

with r as the radial distance from the bioreactor center line. The smallest scale of turbulence is found at $2r d_{\text{imp}}^{-1} = 1$, resulting in a decrease of Φ with increasing distance to the impeller tip. Moreover, Φ varies substantially with the impeller-to-reactor diameter ratio $d_{\text{imp}} d_{\text{STR}}^{-1}$, but less with the impeller type (Nienow, 2021). It is most importantly independent of scale and also found in literature with lower values as 100, 40 or approx. 14 (Nienow et al., 2013; Sieck et al., 2013; Nienow,

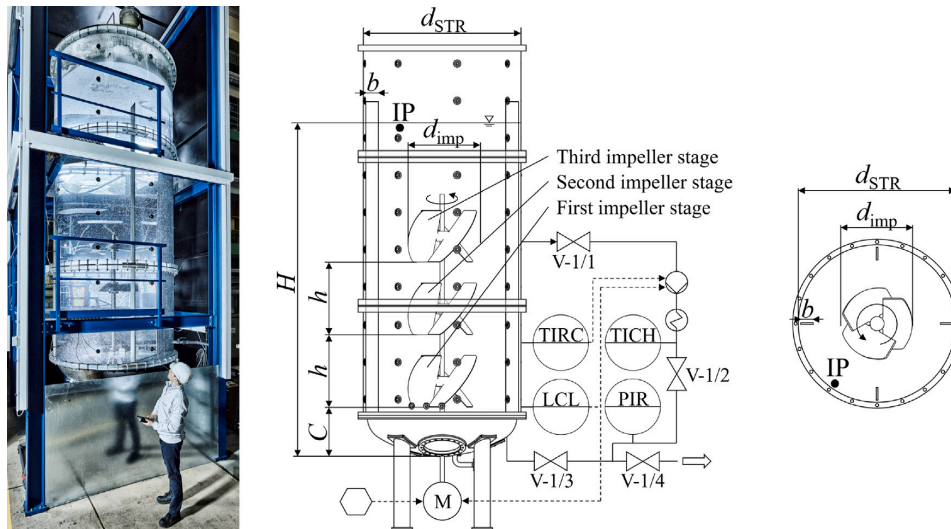


Fig. 1. Schematic overview of the acrylic glass reactor with a filling level H , a diameter d_{STR} , equipped with baffles with a width b . Three Elephant Ear (EE) impellers with a diameter d_{imp} are installed with a spacing h and an off-bottom clearance C . Valves V-1/1, V-1/2 and V-1/3 build a closed loop when heating the reactor. Valve V-1/4 is used to take a sample. Temperature (T), level (L) and pressure (P) are controlled (C), recorded (R) or indicated (I) via static probes. The location of the injection point (IP) for hydrochloric acid for the mixing time experiments is indicated with a black dot.

Table 2

Overview of stirrer Reynolds numbers Re_{STR} , volumetric power inputs $P V^{-1}$, Kolmogorov length scales λ_K , estimated minimum Kolmogorov length scales in the vicinity of the impeller tip $\lambda_{K,\phi=115}$ and estimated Taylor scales L_E for both medium types, deionized water (DW) and isotonic solution (IS).

Medium	Mixing parameter	Impeller frequency n/rpm					
		15	20	25	30	35	40
DW	$Re_{STR} \cdot (10^5)/-$	2.2	2.9	3.7	4.4	5.1	5.9
	$P V^{-1} / W m^{-3}$	4.2	10.0	19.6	33.9	53.9	80.4
	$\lambda_K \cdot (10^{-6}) / m$	119.4	96.2	81.4	71.0	63.2	57.2
	$\lambda_{K,\phi=115} \cdot (10^{-6}) / m$	36.5	29.4	24.9	21.7	19.3	17.5
	$L_E \cdot (10^{-3}) / m$	41.7	36.1	32.3	29.5	27.3	25.5
IS	$Re_{STR} \cdot (10^5)/-$	3.0	4.0	5.0	5.9	6.9	7.9
	$P V^{-1} / W m^{-3}$	4.3	10.1	19.8	34.3	54.4	81.2
	$\lambda_K \cdot (10^{-6}) / m$	95.3	76.8	64.9	56.6	50.5	45.6
	$\lambda_{K,\phi=115} \cdot (10^{-6}) / m$	29.1	23.5	19.8	17.3	15.4	13.9
	$L_E \cdot (10^{-3}) / m$	35.8	31.0	27.8	25.3	23.5	21.9

2021). According to previous studies, no cell damage is likely to occur in the STR utilized herein, since almost all estimated Kolmogorov scales $\lambda_{K,\phi=115}$ are greater than estimated mammalian cell sizes (CHO cell sizes range between 12 μm and 14 μm) and smaller than those reported in literature (Nienow, 2006, 2021; Nienow et al., 2016; Nienow, 2014).

2.2. Lagrangian Sensor Particles

2.2.1. Hardware components

The Lagrangian Sensor Particles (LSPs), developed by the Helmholtz-Zentrum Dresden-Rossendorf (HZDR), have a diameter of $d_{LSP} = 40$ mm, carry an approx. weight of $m_{LSP} = 33$ g and consist of two half-shells, which are connected by a threaded rod and sealed with an O-ring (Fig. 2). Hardware and electronic components are described by Buntkiel et al. (2023a,b), while this study solely makes use of the pressure sensor data at a sample rate of approx. $f = 50$ Hz. For each stirring speed, four LSP datasets are considered, whereby two originate from a separate trial accounting for a total measurement time of at least 245 min (Hofmann et al., 2023).

For the experiments presented, two temperatures ($T \in \{20, 37\}^\circ C$), and thus respective medium properties (DW and IS) are covered (Table 1) to match and demonstrate the technical feasibility for the LSPs during generic fermentation conditions. Therefore, the rpm cases $n \in \{15, 25, 35\}$ rpm are evaluated in DW at $T = 20^\circ C$, and $n \in \{20, 30, 40\}$ rpm are evaluated in IS at $T = 37^\circ C$. In order to be able to compare both datasets quantitatively, the 35 rpm case is evaluated and compared for both conditions and temperatures.

2.2.2. Density calibration

In order to calibrate the LSP densities for each trial, two glass vessels are prepared. As a reference, the first contains the reactor media, obtained from the bottom valve V-1/4. The second one contains also reactor medium, however, is adjusted with additional sodium chloride to match the upper limit of the allowed density range (see Table 1). By submerging the vessels in a water bath, they are kept at the same temperature as the respective reactor medium. If an LSP sinks in the first vessel and surfaces in the second vessel, the LSP's density fulfills the criteria and is used for the trial. If this is not the case, the LSP's density is adjusted by turning the upper half shell, consequently increasing or decreasing the LSP's volume.

2.3. Stokes number estimations

Previous studies (Hofmann et al., 2022; Bisgaard et al., 2021a) utilize the Stokes number St as a characteristic dimensionless number to estimate the flow-following capability of an LSP with regard to the surrounding fluid flow. Findings in literature indicate that a particle can serve as an effective flow-follower if the magnitude of the Stokes number $St \sim \mathcal{O}(10^{-2})$ is met, particularly when investigating single-point flow statistics (Ouellette et al., 2008). Additionally, Tropea et al. (2007) suggest that if $St < 0.1$, the respective particle yields an acceptable level of accuracy in flow tracing, resulting in errors of less than 1 %. Generally, the Stokes number

$$St = \frac{\tau_p}{\tau_f} \quad (6)$$

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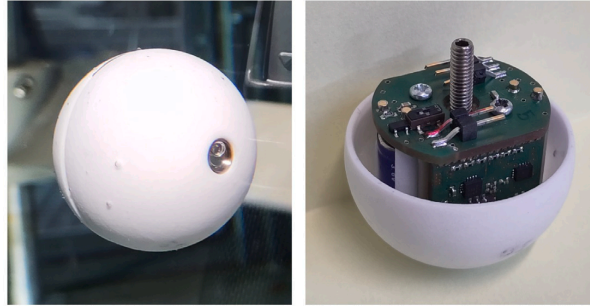


Fig. 2. Closed LSP in a tank (left) and open LSP with hardware components and threaded rod (right) (Buntkiel et al., 2023a).

is defined as the ratio of the particle response time τ_p to the temporal scale of change of the fluid velocity τ_f (Crowe et al., 2011). Based on the single equations for

$$\tau_p = \frac{4}{3} \frac{\rho_p d_p^2}{\eta c_D Re_p}, \quad (7)$$

with ρ_p being the particle density, d_p the particle diameter, η the dynamic viscosity, c_D the drag coefficient according Haider and Levenspiel (Haider and Levenspiel, 1989) (using the four-parameter general drag correlation for a sphere with a sphericity $\phi = 1$), the particle Reynolds number

$$Re_p = \frac{\rho_f d_p |u - v|}{\eta}, \quad (8)$$

which considers the difference between the fluid velocity u and the particle velocity v , and

$$\tau_f = \frac{L}{u}, \quad (9)$$

where L is a characteristic fluid flow length scale, the equation

$$St = \frac{4}{3} \frac{\rho_p}{\rho_f} \frac{d_p}{|u - v| c_D} \frac{u}{L} \quad (10)$$

can be derived.

Regarding the definition of τ_f , instead of using $L = d_{imp}$ as proposed by Reinecke et al. (2012), which contemplates the meso-scale of the mixing process, but would, however, underestimate the Stokes number as demonstrated by Hofmann et al. (2022), this study makes use of the approach that $L = L_E$ with the Taylor scale

$$L_E = u_0 \sqrt{\frac{15\nu}{\bar{\epsilon}_T}} \quad (11)$$

to cover the Stokes number estimation on the micro-scale. Therein, ν is the kinematic viscosity and u_0 , defined as the root-mean-square of the fluid velocity (Huillier, 2021), is in this case estimated as the tip speed u_{tip} . Moreover, as described in Reinecke et al. the fluid velocity u is estimated as the tip speed u_{tip} so that

$$St = \frac{4}{3} \frac{\rho_p}{\rho_f} \frac{d_p}{\left(u_{tip} - |\bar{v}|_{z,max}\right) c_D} \sqrt{\frac{\bar{\epsilon}_T}{15\nu}} \quad (12)$$

is derived, where $|\bar{v}|_{z,max}$ is the maximum average axial LSP velocity for the respective impeller frequency at height z . This approach covers especially the most turbulent region near the impeller tip, whereas the Stokes number for the macro-scale can be represented if the overall circulation time is considered as $\tau_f = \bar{\tau}_{circ,overall}$ (Reinecke et al., 2012).

2.4. Vertical probability of presence and axial velocities

After assessing the flow-following capability of the LSPs in the utilized reactor volume, the vertical probability of presence and axial

velocities are calculated. To do so, the vertical position of an LSP is obtained by its hydrostatic pressure measurement by applying Pascal's law

$$z(t) = H - \frac{p(t) - p_0}{\rho_f g}, \quad (13)$$

where $z(t)$ is the LSP's height at a point in time, H is set as the filling level of the reactor, $p(t)$ is the measured pressure at a point in time, p_0 is the measured ambient pressure before each trial for each LSP, ρ_f is the density of the respective fluid and g the gravitational acceleration. All evaluated raw data is pre-processed with a low-pass filter and interpolated afterwards. For this purpose, a moving average of five consecutive values and a spline interpolation to obtain a uniform 50 Hz signal are used, respectively. For further analyses, the reactor is divided into 42 equally distributed vertical reactor volumes j with a respective height of $h_j = 0.1$ m.

The vertical probability of presence P is generally calculated in relative percentage as

$$P_j = 100 \cdot \frac{N_j}{M} \quad (14)$$

with N_j being the number of counts in the respective bin j and M being the total number of counts in the dataset.

The LSP's axial velocity $v_z(t)$ is the time derivative of the LSP's vertical position with respect to the interpolated 50 Hz ($dt = 20$ ms) signal as

$$v_z(t) = \frac{dz(t)}{dt}. \quad (15)$$

The velocity can be distinguished between positive $v_{z,pos}(t)$ (upwards movement) and negative $v_{z,neg}(t)$ (downwards movement) velocity. For each, average values and corresponding uncertainties Δ are generally calculated as

$$4_{\bar{x}} = \sqrt{\frac{1}{N(N-1)} \sum_{i=1}^N (x_i - \bar{x})^2} \quad (16)$$

with N being the sample size and $\bar{x}$ the average value. Due to differing amounts of initially obtained data points per dataset, a weighted average with a corresponding error propagation is calculated and plotted.

2.5. Determination of circulation time distributions

The tracer injection method stands as the predominant approach for acquiring circulation times and specifically mixing times. This method involves introducing a tracer substance into the liquid, followed by the analysis of the rate at which uniform blending occurs. However, in order to attain detailed information about dead zones, overall flow structures, and the probability density of the circulation time distribution (CTD) more information is required. This is the reason why the pressure sensor data of LSPs is utilized. In previous publications, only one reactor height was chosen to determine a CTD, either with a

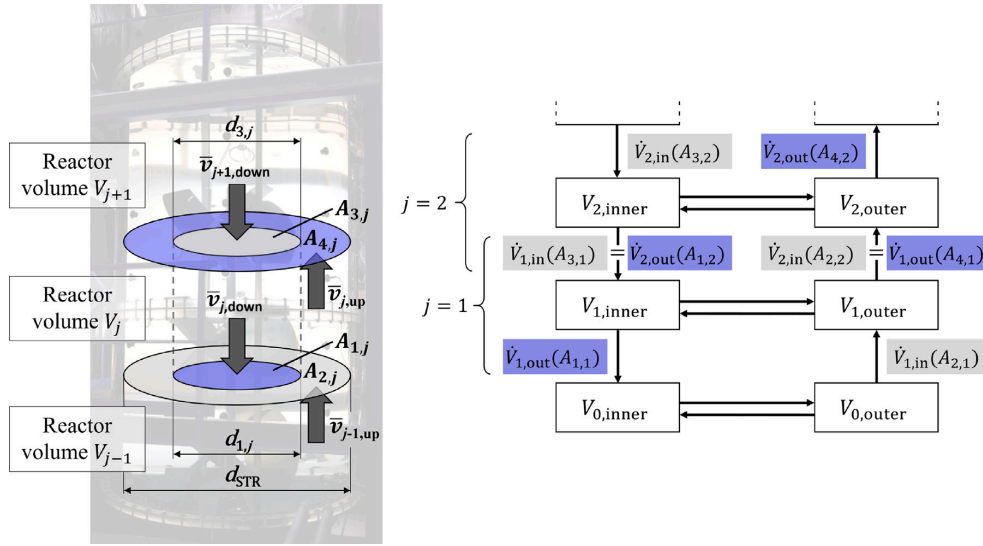


Fig. 3. Schematic depiction of the residence time modeling approach and the coherence between the respective reactor volumes and their volume flow rates.

physically installed aerial ring (Middleton, 1979) or deliberately chosen at the height of the impeller (Bisgaard et al., 2021a). However, in order to hydrodynamically characterize the entire reactor and determine compartments, an overall picture is required and herein evaluated. Hence, CTDs are obtained for each reactor slice j over the entire reactor height H (Reinecke, 2013).

To do so, as previously described, the reactor is divided into 42 equally distributed vertical reactor volumes j with their vertical central plane. If an LSP crosses a respective central plane at $t = 0$ from top to bottom, the signal is recorded as $t_{\text{circ},j,t=0}$. Once it crosses that plane the second time from top to bottom with the signal $t_{\text{circ},j,t>0}$, the time difference $t_{\text{circ},j,\text{dt}}$ is referred to one circulation time value per plane j . This method yields a distribution for all reactor planes and discloses axial hydrodynamic compartments throughout the reactor.

2.6. Determination of residence time distributions

In order to reveal the hydraulic residence time distributions and present a second approach to distinguish hydrodynamic compartments within the reactor, several assumptions are made, based on the descriptions in Fig. 3:

- Although the reactor is equipped with a dished head, it is assumed to be an overall cylinder with a surface area of $A = 3.14 \text{ m}^2$. With the height of $h_j = 0.1 \text{ m}$ each volume amounts to $V_j = 0.314 \text{ m}^3$.
- Due to the predominant axial flow direction of the EE impellers, in each reactor volume V_j , an inner volume $V_{j,\text{inner}}$ and an outer volume $V_{j,\text{outer}}$ is assumed, which shows a downflow and an up-flow behavior, respectively. Hence, each individually recorded LSP velocity data point within a reactor volume is allocated either to the inner volume as $v_{j,\text{down}}$, if it shows a negative value (meaning positive pressure change over time), or allocated to the outer volume as $v_{j,\text{up}}$, if it shows a positive value (meaning negative pressure change over time). For each reactor volume, respective weighted time averaged values $\bar{v}_{j,\text{down}}$ and $\bar{v}_{j,\text{up}}$ are determined.
- Each reactor volume has a lower boundary ($A_{\text{STR}} = A_{1,j} + A_{2,j}$) and an upper boundary ($A_{\text{STR}} = A_{3,j} + A_{4,j}$), which are defined respectively as

$$A_{1,j} = \frac{\pi}{4} \cdot d_{1,j}^2; A_{2,j} = \frac{\pi}{4} (d_{\text{STR}}^2 - d_{1,j}^2) \quad (17)$$

$$A_{3,j} = \frac{\pi}{4} \cdot d_{3,j}^2; A_{4,j} = \frac{\pi}{4} (d_{\text{STR}}^2 - d_{3,j}^2), \quad (18)$$

where $A_{1,j}$ and $A_{3,j}$ can be different. Therefore, depending on their respective size, the inner volume $V_{j,\text{inner}}$ has either a cylindrical or a tapered shape.

- Due to mass conservation $d\rho \, dr^{-1} = \text{const.}$, it can be generally concluded that the volume flow into a reactor volume $\dot{V}_{j,\text{in}}$ must be equal to the volume flow out of that volume $\dot{V}_{j,\text{out}}$ as

$$\dot{V}_{j,\text{in}} = \dot{V}_{j,\text{out}}, \quad (19)$$

where they can be calculated as

$$\dot{V}_{j,\text{in}} = |\bar{v}|_{j+1,\text{down}} \cdot A_{3,j} + \bar{v}_{j-1,\text{up}} \cdot A_{2,j} \quad (20)$$

$$\dot{V}_{j,\text{out}} = |\bar{v}|_{j,\text{down}} \cdot A_{1,j} + \bar{v}_{j,\text{up}} \cdot A_{4,j}, \quad (21)$$

leading to

$$|\bar{v}|_{j+1,\text{down}} \cdot A_{3,j} + \bar{v}_{j-1,\text{up}} \cdot \frac{\pi}{4} (d_{\text{STR}}^2 - d_{1,j}^2) = |\bar{v}|_{j,\text{down}} \cdot A_{1,j} + \bar{v}_{j,\text{up}} \cdot \frac{\pi}{4} (d_{\text{STR}}^2 - d_{3,j}^2). \quad (22)$$

Since d_1 and d_3 are unknown, an iteration takes place, which starts with the lowest reactor volume and an assumed start value for d_1 . Consequently, d_3 can be calculated as

$$d_{3,j} = \sqrt{\frac{d_{1,j}^2 \cdot (|\bar{v}|_{j,\text{down}} + \bar{v}_{j-1,\text{up}}) + d_{\text{STR}}^2 \cdot (\bar{v}_{j,\text{up}} - \bar{v}_{j-1,\text{up}})}{|\bar{v}|_{j+1,\text{down}} + \bar{v}_{j,\text{up}}}}. \quad (23)$$

- With continuing the calculation of the respective diameters for $j = 1$ towards the next reactor volume $j = 2$ and so forth (see Fig. 3), the previously calculated value for $d_3(j = 1)$ must be equal to $d_1(j = 2)$. Hence, the calculated top surface area (e.g. $A_{3,1}$) from the previous step must be equal to the bottom surface area (e.g. $A_{1,2}$) in the current step as

$$d_{3,j} = d_{1,j+1} \Leftrightarrow A_{3,j} = A_{1,j+1} \quad (24)$$

- If Eq. (19) is not met, the iteration process restarts at the lowest reactor volume. The start value is preset to $d_1 = 0.20 \text{ m}$, the iteration step width is set at 0.01 m , and the maximum value $d_1 = 1.80 \text{ m}$. If an iteration loop ends successfully with the upmost possible reactor volume $V_{j=40}$, the values are saved.

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- (vii) There is a possibility that side flows can occur, and thus the inner and outer volumes in a reactor volume j can exchange mass. Furthermore, as a boundary condition, the lowermost volume $j = 0$ and the topmost volume $j = 41$ do neither interact with the dished head of the reactor nor with the gas volume above the liquid surface, respectively.

Eventually, based on those results, inner and outer volume flows for each reactor volume can be calculated as

$$\bar{V}_{j,\text{inner}} = |\bar{v}|_{j+1,\text{down}} \cdot \bar{A}_{3,j}, \quad (25)$$

$$\bar{V}_{j,\text{outer}} = \bar{v}_{j-1,\text{up}} \cdot \bar{A}_{2,j}, \quad (26)$$

which is finally checked for the overall mass conservation criteria, meaning

$$\sum_{j=1}^{40} \bar{V}_{j,\text{inner}} \approx \sum_{j=1}^{40} \bar{V}_{j,\text{outer}}. \quad (27)$$

If this is the case, the respective volume flows are taken into account for an average value and the respective uncertainty calculated on error propagation, leading to the respective hydraulic residence times as

$$\bar{\tau}_{j,\text{inner}} = \frac{\bar{V}_{j,\text{inner}}}{\bar{V}_{j,\text{inner}}} \quad \text{and} \quad \bar{\tau}_{j,\text{outer}} = \frac{\bar{V}_{j,\text{outer}}}{\bar{V}_{j,\text{outer}}}. \quad (28)$$

For an additional assessment, respective residence times are added up to calculate the overall average hydraulic residence time as

$$\bar{\tau}_{\text{overall}} = \sum_{j=1}^{40} \bar{\tau}_{j,\text{inner}} + \sum_{j=1}^{40} \bar{\tau}_{j,\text{outer}}. \quad (29)$$

This approach treats the STR as if it would be consisting of two interconnected plug flow reactors for the inner and for the outer volume flow, respectively.

2.7. Compartment determination and Lagrangian regime analysis

Based on previously described calculations, hydrodynamic compartments are determined for both the circulation time and residence time distributions. To do so, in order to present a quantitative approach, the respective circulation time and residence time trends are first smoothed with a *moving average* of five consecutive values. Afterwards, each single value for the reactor slice j in a trend is compared with the values $j-2$ and $j+2$ in the same trend. A deliberate cut-off factor of 2.3 shows the best fitting results for the investigated rpm cases, to depict a division between centrally located rather low values and comparatively high values at the top and bottom of the reactor.

The primary difficulty accompanied with scale-down simulators lies in creating a simulator on laboratory scale, which accurately replicates the conditions of the industrial process (Gaugler et al., 2022; Neubauer and Junne, 2016). Presently, microbial lifelines (Lapin et al., 2004, 2006) are primarily obtained by means of computational fluid dynamic models. Blöbaum et al. (2023) reviewed these concepts critically and describe therein an approach of Haringa et al. where different Euler–Lagrange analysis methods are utilized to gain insight about metabolic dynamics in fermentation processes on industrial scales (Haringa et al., 2017, 2018; Haringa, 2023). Based on numerically determined lifelines in a domain with three zones, in which different metabolic responses occur, a metabolic Lagrangian regime analysis offers valuable information about the average regime residence time of cells. This can be further used to adjust a scale-down simulator. This study, however, comprises the hydrodynamic Lagrangian regime analysis with obtained LSP data by encompassing on the one hand the calculated axial locations of the hydrodynamic compartment barriers, based on the circulation time distribution results, for the respective rpm case. On the other hand, the LSP's Lagrangian axial lifeline over time $h(t)$ is utilized. Similar to the approach by Haringa et al. (2018), a residence

time distribution per hydrodynamic regime (in this study: upper, center and lower) is obtained for any LSP lifeline. Hence, a percentage of cells exposed to theoretical oxygen deprivation conditions in the upper compartment can be derived, although this study only utilizes and analyses the single-phase hydrodynamics.

2.8. Mixing time measurements

The global mixing time $t_{\text{mix,global,95}}$ is determined experimentally (Fitschen et al., 2021b) in the same reactor by means of the decoloration method utilizing bromothymol blue (BTB) and the addition of a 2 M hydrochloric acid (HCl) and a 2 M sodium hydroxide (NaOH) solution in DW at $T = 37^\circ\text{C}$. Initially, a 30 g L^{-1} BTB solution in 99.9 v/v-% Ethanol is prepared, of which 100 mL is added into the reactor volume. Prior to each measurement, pH is first adjusted to $7.0 < \text{pH} < 7.5$ and checked with a WTW Sentix 41-3 probe, which corresponds to a green color. Consequently, 250 mL of the NaOH solution is added in order to gain a color shift to blue. After this preparation step, 500 mL of HCl solution is pneumatically introduced from a storage tank (1.2 m above the water surface) into the reactor to achieve a color shift to yellow via green. Afterwards, the storage tank is rinsed with DW. Simultaneously, as an indicator to start the measurement, the background illumination is switched on at the moment of the addition (Rosseburg et al., 2018). The injection point (IP, see Fig. 1) is located at a distance of 0.1 m from the reactor wall and 35° before the baffle in rotational direction of the impeller blades. The injection of HCl takes 3 s and is performed 0.2 m beneath the water surface through a vertically installed diffusor nozzle outlet with a diameter of 30 mm and a height of 50 mm. Uniform lighting condition is provided by five spotlights, four white LED panels and a white diffuser foil, which is located behind the reactor. The decoloration is recorded at a sampling rate of 50 Hz by a NIKON D7500 with a SIGMA lens (1:1.8, 20 mm f/22.0) at a resolution of 1920×1080 pixels at a height of 0.9 m above the lowest point of the reactor and a distance of 5.1 m to the impeller shaft. The mixing is quantitatively analyzed with an in-house developed open-source MATLAB algorithm (Fitschen et al., 2021a) utilizing the evolution of the gray values over time. The global mixing time is defined as the duration starting from the HCl solution addition until the normalized mean gray value attains 95 % of the final value (Rosseburg et al., 2018). Each experiment is repeated three times at each $n \in \{16, 21, 29, 37, 40\}$ rpm.

2.9. Flow number estimations

Furthermore, in order to characterize a specific impeller type, describe the flow phenomena in an STR and provide crucial parameters for modeling, four different Flow numbers are estimated and introduced in this study: (i) the Pumping number $N_{Q,p}$, (ii) the Circulation Flow number $N_{Q,c}$, (iii) the Residence Flow number $N_{Q,r}$ and (iv) the Exchange Flow number $N_{Q,e}$ (Vrábel et al., 2000, 1999; Nienow, 1998; Schubert, 2008). The Residence Flow number is not found in literature and introduced herein as a measure to describe the overall flow of LSPs in the STR. Being independent of the impeller frequency in the turbulent regime, they proof to be valuable for process scaling.

- (i) The Pumping number $N_{Q,p}$ for an axial down-pumping impeller is generally calculated by the swept volume flow Q_p through the impeller zone d_{imp} (Tattersson, 1991) at the impeller frequency n as

$$N_{Q,p} = \frac{Q_p}{n \cdot d_{\text{imp}}^3}. \quad (30)$$

- (ii) The circulation volume flow Q_c considers an additional entrained flow (Schubert, 2008). It is influenced by the introduction of the overall momentum flow into the fluid and utilizes the average circulation rate in the whole path (Vrábel et al., 1999). In case of

a single-phase flow as investigated in this study, the Circulation Flow number is defined as

$$N_{Q,c} = \frac{Q_c}{n \cdot d_{imp}^3} \quad (31)$$

As an approximation, the reactor volume V is pumped within an average circulation time $\bar{t}_{circ}$, which is described by

$$\bar{t}_{circ} = \frac{V}{Q_c} \quad (32)$$

and consequently, both Eqs. (30) and (31) yield the expression

$$\frac{1}{\bar{t}_{circ}} = \underbrace{\frac{N_{Q,c} \cdot d_{imp}^3}{V}}_{k_{to}} \cdot n = k_{to} \cdot n, \quad (33)$$

meaning the average circulation time is inversely proportional to the impeller frequency ($\bar{t}_{circ}^{-1} \propto n$) (Middleton, 1979). This plot reveals the turn-over constant k_{to} as the slope, after which $N_{Q,c}$ is derived.

- (iii) To calculate the Residence Flow number $N_{Q,r}$ the same approach is used, however, instead of taking the average circulation time into account, all residence times $\tau_{j,inner}$ and $\tau_{j,outer}$ are added up to obtain the total turn-over time.
- (iv) The Exchange Flow number

$$N_{Q,e} = \frac{Q_e}{n \cdot d_{imp}^3} \quad (34)$$

describes the exchange flow rate Q_e as a result of the flow driven by turbulence across the cross-sectional area of the reactor (Vrábel et al., 2000). Hence, it specifies the magnitude of fluid exchanged between neighboring compartments in axial direction (Vrábel et al., 1999). Since volume flows are determined in the course of the residence time calculations, this Flow number can be directly calculated for different reactor heights without a reciprocal regression.

3. Results and discussion

In this chapter, the evaluation of the pressure sensor data is described, depicted and discussed. According to the calculations explained in the previous chapter, the Lagrangian Sensor Particle's (LSP) vertical probability of presence, their resulting axial velocity in the reactor and their corresponding circulation time and residence time distribution is presented in order to determine hydrodynamic compartments. Moreover, a Lagrangian regime analysis is presented to estimate potential oxygen depletion periods for CHO cells on industrial scale. Dimensionless numbers as the Stokes number and different Flow numbers are estimated to put the results in context with literature. Eventually, measured global mixing times in the same reactor are compared to previously acquired results and literature correlations.

3.1. Stokes number estimations

In order to estimate and eventually compare the flow-following capability of the LSPs used in this study by means of the Stokes number St , calculation approaches are suggested in Section 2.3. The values for the micro-scale in the vicinity of the EE impellers are estimated as $St \in \{4.0, 4.7, 5.1, 5.4, 5.7, 6.2\}$ for the experiments in DW and $St \in \{4.5, 5.3, 5.9, 6.2, 6.6, 7.2\}$ for the experiments in IS for all impeller frequencies, respectively. Regarding the Stokes number for the meso-scale, values are between $0.17 < St < 0.18$ and for the macro-scale $St \approx 0.004$ (see Table 4). Considering the results described by van Van Barneveld et al. (1987a,b), Reinecke et al. (2012) and Bisgaard et al. (2021a), this study's Stokes numbers are in a similar range.

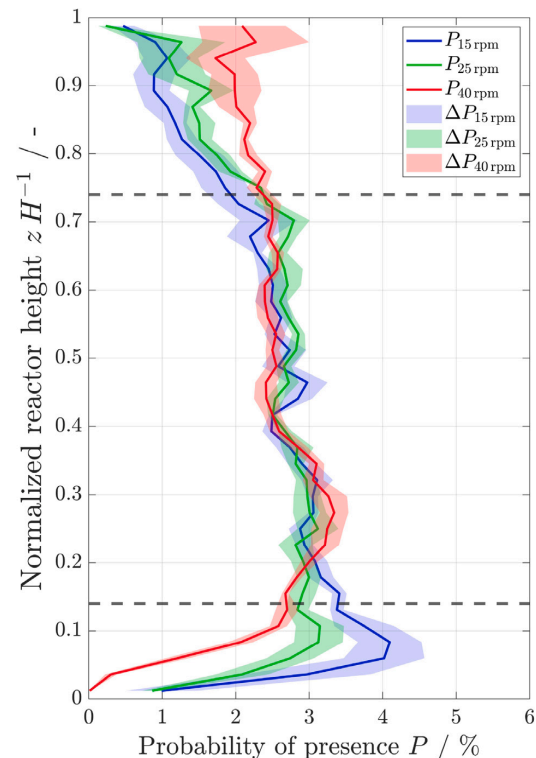


Fig. 4. Axial probability of presence over the normalized reactor height ($H = 4.2$ m) for four LSP data sets for each impeller frequency of 15 (blue line), 25 (green line) and 40 rpm (red line) for a vertical bin size of 10 cm. Shaded areas show uncertainty Δ of respectively colored rpm case. Gray dashed lines indicate the lowest and uppermost edges of EE impeller blades. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Vertical probabilities of presence

Although the LSPs are adjusted close to a neutral buoyancy in the respective medium of use (Table 1), it is of essence to conduct an axial distribution assessment within the entire reactor to evaluate further results. In Fig. 13 (Appendix A), an exemplary axial movement sequence over approx. 1700 s of the LSP (ID 3) of trial no. 3 (LSP3-3) is depicted over the normalized reactor height together with a schematic view of the impeller blade positions. The LSP seems to flow primarily at the height of the impellers, whereas the lower and upper regions are less covered. A total time of at least 245 min is recorded with four LSPs for each rpm case. The resulting axial probability of presence for 15 (blue), 25 (green) and 40 rpm (red) is plotted in Fig. 4 with their respective uncertainties.

Despite their density adjustment for the respective reactor medium, the trend for 15 rpm reveals an accumulation with up to 4% probability of presence at the reactor bottom, up to the lower dashed line, which indicates the lower edge of the first EE impeller stage. Beyond this point, which coincides with the location of the three EE impeller stages, the distribution becomes more uniform, resembling the trend of the 25 and 40 rpm case. Above the upper dashed line, which corresponds to the upper edge of the third EE impeller stage, all probabilities with exception to the 40 rpm case progressively decrease until lower than 1% at the fluid's surface. Generally, the 40 rpm case is axially comparatively best distributed, however, showing a lack of data points at the bottom of the reactor. During the experiments at $T = 37^\circ\text{C}$, it was found that

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the density of the LSPs may decrease to a minimum of $(\rho_f - 1) \text{ kg m}^{-3}$ depending on their operating duration. It is assumed that the enclosed air, which is at room temperature when calibrating, equilibrates to the surrounding temperature in the medium, and thus leading to an increased pressure inside by a factor of approx. 1.05. As a result, the LSP's volume gradually increases by a theoretical amount of approx. $37 \cdot 10^{-9} \text{ m}^3$. The change of volume proves to be sufficient resulting in a density reduction of $\delta \rho_p \leq 1 \text{ kg m}^{-3}$. Taking the hydrostatic pressure into consideration, this effect should be equalized below the normalized height of $z H^{-1} \approx 0.88$ depending on the stability of the LSP and its sealing components.

Due to the described sensitive density effect, the axial probability of presence over the reactor height is evaluated for the 35 rpm case for both medium types and quantitatively compared in Fig. 14 in Appendix B. It can be observed that the distributions in the center region are similar within their uncertainties for both medium types, in deionized water (DW) and in the isotonic solution (IS). Aside from that, both the bottom and upper region show a slightly increased divergence, respectively, up to 1 %.

3.3. Axial velocity distributions

Based on the flow direction of the LSPs, positive (upwards movement, solid lines) and negative (downwards movement, dashed lines) axial velocities are obtained by using the derivative of the vertical position concerning the recording frequency, which is depicted in Fig. 5.

By plotting three exemplary impeller frequencies as 15 (blue), 25 (green) and 40 rpm (red), an overall consistent pattern can be observed. In case of $|\bar{v}|_{z,\text{neg}}$, two peaks occur at normalized reactor heights of $z H^{-1} \approx 0.20$ and 0.65 . For 25 and 40 rpm, a third peak emerges at $z H^{-1} \approx 0.45$. A single peak is detectable for the trend of $\bar{v}_{z,\text{pos}}$ at $z H^{-1} \approx 0.20$. This behavior can be attributed to the general pumping nature of a multi-stage axial-pumping agitator, whereby all heights match the center of any impeller stage, respectively. For all studied rpm cases, at $z H^{-1} \approx 0.65$, the LSPs reach their respective maximum downward flow velocity of $|\bar{v}|_{z,\text{neg}} \in \{0.21, 0.37, 0.54\} \text{ m s}^{-1}$ due to a strong axial suction behavior of the third impeller stage. This behavior decreases slightly towards the vertical center of the reactor, then increases with higher impeller frequencies at the second stage, and finally increments to a last peak at $z H^{-1} \approx 0.20$. Here, they are pumped either into the less turbulent bottom region of the reactor, where their velocity is distinctively decreased, or are instantly redirected towards the reactor walls and baffles from where they move upwards. The latter is indicated by a single peak in all trends of upwards velocities with $\bar{v}_{z,\text{pos}} \in \{0.18, 0.29, 0.49\} \text{ m s}^{-1}$, respectively. These velocities progressively decrease further, eventually falling below 0.1 m s^{-1} above the third impeller stage, which signifies a similar behavior to that observed in the bottom region. Hence, the fluid flow followed by the LSPs is mainly downwards in the proximity of the impellers and upwards near the reactor wall. The comparatively high uncertainty at 40 rpm for $z H^{-1} < 0.05$ is due to the sparse data points available for evaluation, as seen in Fig. 4.

As shown for the probability of presence, also the LSP's average axial velocity is scrutinized regarding both medium types, DW and IS, at two different temperatures, and depicted in Fig. 15 in Appendix C. Despite the previously described behavior as two peaks for $|\bar{v}|_{z,\text{neg}}$ and one peak for $\bar{v}_{z,\text{pos}}$ at the respective heights, only minor differences occur in the vertical center region of the reactor. However, especially in the reactor bottom region and at the center height of the third impeller stage, velocity differences of $\delta \bar{v}_{z,\text{pos}} = 0.12 \text{ m s}^{-1}$ and $\delta |\bar{v}|_{z,\text{neg}} = 0.05 \text{ m s}^{-1}$ are shown, respectively. Nevertheless, since for both medium types similar trends and values are shown, upcoming evaluations for all rpm cases are directly compared with each other independently of their media type.

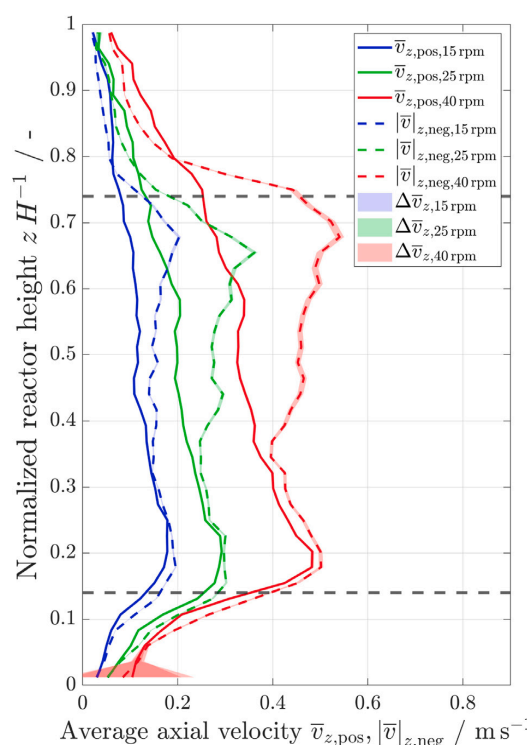


Fig. 5. Average positive (solid lines, upwards flow) and negative (dashed lines, downwards flow) axial velocity distributions over the normalized reactor height ($H = 4.2 \text{ m}$) for four LSP data sets for each impeller frequency as 15 (blue), 25 (green) and 40 rpm (red) for a vertical bin size of 10 cm. Shaded areas show uncertainty Δ of respectively colored data set. Gray dashed lines indicate the lowest and uppermost edges of EE impeller blades. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Circulation time distributions

The circulation time $t_{\text{circ},z}$ of an LSP is referred to the axially central plane of the reactor volume j at the height z and is determined by means of its passage time difference from top to bottom through that plane. Hence, this method yields a circulation time distribution (CTD) per height z .

An exemplary illustration of a CTD is shown in Fig. 6, for the 15 rpm case at a normalized reactor height of $z H^{-1} = 0.22$, which constitutes for the axial center of the first impeller stage. As previous studies have shown (Middleton, 1979; Bisgaard et al., 2021a; Mann et al., 1981; Reinecke, 2013), the CTD is log-normally distributed, as long as sufficient data points are obtained. In Fig. 6, a total of 441 single circulation time values are utilized, which are stacked above each other in a bar plot. As a reference, the mean value ($t = 34.9 \text{ s}$, light gray dotted line), the median value ($t = 20.9 \text{ s}$, gray dash-dotted line), and the weighted average value ($t = 35.2 \text{ s}$, dark gray dashed line) are shown. However, due to a partly sparse availability of data points at the bottom and top of the reactor, large values may severely influence the average value, albeit not being necessarily representative. Consequently, presuming a distribution with at least 400 data points per bin as significant as shown in Fig. 6, for the normalized reactor height of e.g. $z H^{-1} = 0.86$, which is located in the center between the upper edge of the third impeller stage and the media surface, it would require a 10-fold longer recording time (approx. 11 h with four simultaneously used LSPs), owed to a total of 46 data points for the

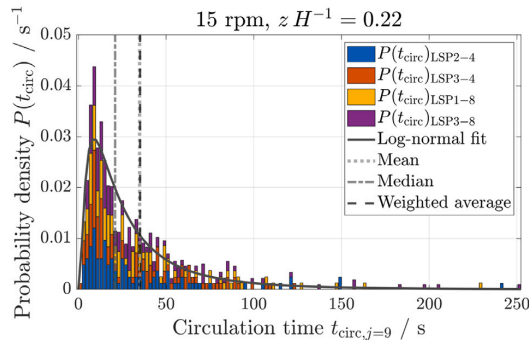


Fig. 6. Exemplary probability density $P(t_{\text{circ}})$ of the circulation time distribution for all four LSPs (ID 2 and ID 3 of experiment no. 4, ID 1 and ID 3 of experiment no. 8) at 15 rpm and a normalized reactor height of $zH^{-1} = 0.22$. A log-normal fit (black curve), the mean ($t = 34.9$ s, light gray dotted line), median ($t = 20.9$ s, gray dash-dotted line) and weighted average ($t = 35.2$ s, dark gray dashed line) are depicted based on 441 data points. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

respective bin. Due to differing recording lengths of the four utilized LSPs, the weighted average is chosen for representing $\bar{t}_{\text{circ}}$ for the corresponding reactor height z .

By plotting those weighted average values $\bar{t}_{\text{circ},z}$ over the normalized reactor height in Fig. 7, an overall insight into the axial homogeneity of the macromixing process can be attained (Bajpai and Reuss, 1982). Moreover, one turn-over point is determined for each rpm case, which constitutes to the reactor height z of the shortest average circulation time as described by Reinecke (2013). For all impeller frequencies, this plane is between $0.19 < zH^{-1} < 0.24$, constituting the vertical center of the first impeller stage, with values of $\bar{t}_{\text{circ},z} \in \{34.6, 22.2, 13.2\}$ s, for 15, 25 and 40 rpm, respectively. Notably, the circulation times exhibit a relatively shallow trend within this range, expanding over a centrally located regime between $0.15 \lesssim zH^{-1} \lesssim 0.75$. Beyond this, a strong increase in circulation times and their uncertainties mark both a top and a bottom regime with approx. 10-fold higher values. High uncertainties are attributed to the lack of data points in respective bins as previously described.

With the approach described in Section 2.7, quantitative regime zoning provides information about hydrodynamic compartments and their distinct boundaries, which are situated in the vicinity of the lower and upper edge of the first and third impeller stage, respectively (gray dashed lines). Furthermore, a respective average overall circulation time $\bar{t}_{\text{circ,overall}}$ can be calculated by means of the weighted average, which is (61.8 ± 2.8) s for 15 rpm, (38.2 ± 1.4) s for 25 rpm and (24.2 ± 0.7) s for 40 rpm (see Table 4).

3.5. Residence time distributions

Utilizing the calculation approach described in Section 2.6, average hydraulic residence time distributions (RTD) are presented in Fig. 8 for impeller frequencies as 15 (blue), 25 (green) and 40 rpm (red) over the normalized reactor height. Essentially, these values describe the average time of an LSP within a reactor volume V_j , based on its average velocity and consequently its direction as $|\bar{v}|_{j,\text{down}}$ or $|\bar{v}|_{j,\text{up}}$. This information further specifies whether the LSP is assumed to be located in the inner $V_{j,\text{inner}}$ or in the outer $V_{j,\text{outer}}$ reactor volume, respectively. Hence, the average inner $\bar{t}_{\text{inner},z}$ and outer $\bar{t}_{\text{outer},z}$ residence time is derived.

Similar to the trends shown in Fig. 7, three characteristic regimes are calculated for each impeller frequency: a center regime with comparatively low values at the height of the EE impeller stages, and a top

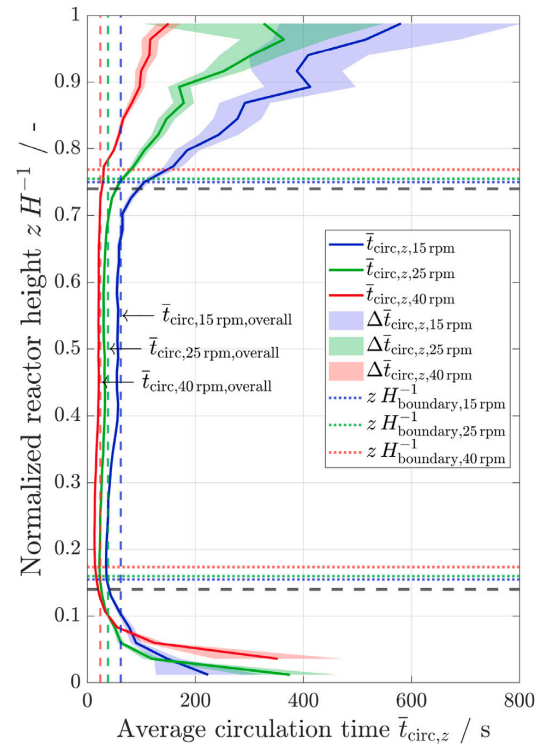


Fig. 7. Average circulation time distribution over the normalized reactor height ($H = 4.2$ m) for four LSP data sets for each impeller frequency of 15 (blue), 25 (green) and 40 rpm (red) for a vertical bin size of 10 cm. Shaded areas show uncertainty Δ , horizontal dotted lines show hydrodynamic compartment boundaries (see Section 2.7) and vertical dashed lines show the overall weighted average value of the respectively colored data set. Gray dashed lines indicate the lowest and upmost edges of EE impeller blades. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and a bottom regime with higher values. Generally, in the vertical center and in the reactor bottom, residence times in the inner volume are lower than those in the outer volume, meaning a more rapid passage in the vicinity of the impellers or below than near the reactor wall. Nevertheless, between a normalized reactor height of $0.2 < zH^{-1} < 0.35$ both trends tend to approach similar values at $\bar{t} \in \{0.2, 0.4, 0.6\}$ s or cross each other so that $\bar{t}_{\text{inner}} > \bar{t}_{\text{outer}}$. Moreover, when comparing the latter observation with the average axial velocity distribution results in Fig. 5 at the same height, where also both upwards and downwards velocity values almost align, it can be deduced that in this region a comparatively high turbulence occurs. The underlying data regarding the calculated surface areas of the respective reactor volumes (not shown here) demonstrate for both upwards and downwards flows a similar area ($A_1 \approx A_2 \approx 0.5 \cdot A_{\text{STR}}$). Hence, most likely induced by the emerging stagnation pressure in the rather less turbulent bottom regime, the first impeller stage exhibits an increased radial pumping character. This effect could only be estimated if solely the data of the CTD were available, since the separation of the flows and resulting residence times could not be resolved. Yet, the CTD confirms the described effect by showing the lowest average circulation times in this region (Fig. 7). Furthermore, it can be observed that just above the third EE impeller stage, at $zH^{-1} \approx 0.75$, the respective trends of the inner and outer residence times cross and show $\bar{t}_{\text{inner}} > \bar{t}_{\text{outer}}$. Similarly to Fig. 5, where respective positive and negative velocity trends also cross at the height, the upper regime generally demonstrates high residence times

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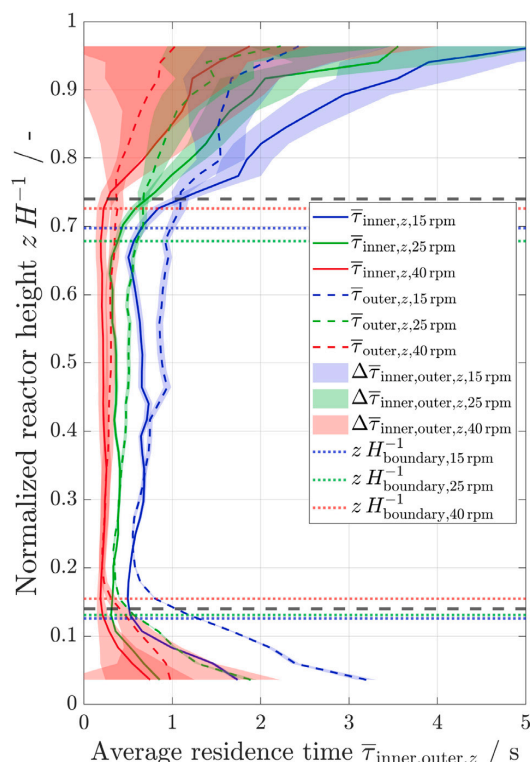


Fig. 8. Average hydraulic residence time distribution for both the inner $V_{j,inner}$ and outer reactor volume $V_{j,outer}$ over the normalized reactor height ($H = 4.2\text{ m}$) for four LSP data sets for each impeller frequency as 15 (blue), 25 (green) and 40 rpm (red) for a vertical bin size of 10 cm. Shaded areas show uncertainty Δ and horizontal dotted lines show hydrodynamic compartment boundaries (see Section 2.7) with respect to the inner residence time of the respectively colored data set. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with comparatively high uncertainties due to the lack of data points as described in previous sections. For further evaluations, these residence times are added up respectively in order to obtain the overall added hydraulic residence time (see Eq. (29)) and are stated in Table 4.

3.6. Lagrangian regime analysis

For the first time, LSP data provides emerging advancements with respect to an experimental, hydrodynamic Lagrangian regime analysis on industrial scale. This study does not focus on metabolic regimes, as demonstrated in Haringa et al. for an aerated yeast fermentation in a 22 m³ fermentor (Haringa et al., 2017, 2018), but on single-phase hydrodynamic regimes. As described and depicted in Section 3.4, the compartment barriers are calculated based on the circulation time distribution and hereinafter utilized by discretizing the LSP's axial lifeline into three regimes. Fig. 9 depicts the residence time distribution (RTD) for the impeller frequencies 15 and 40 rpm per hydrodynamic regime τ_{regime} (upper (red), center (green) and lower (blue) compartment).

Intricate flow patterns emerge in shown compartments, specifically the RTD for 40 rpm demonstrates the characteristic exponential decay of a recirculation flow as already demonstrated by Haringa et al. (2018). They show a similar distribution behavior, although a comparatively dense amount of data from numerical simulations is used, in combination with a four-staged, six-bladed Rushton turbine (RT-6)

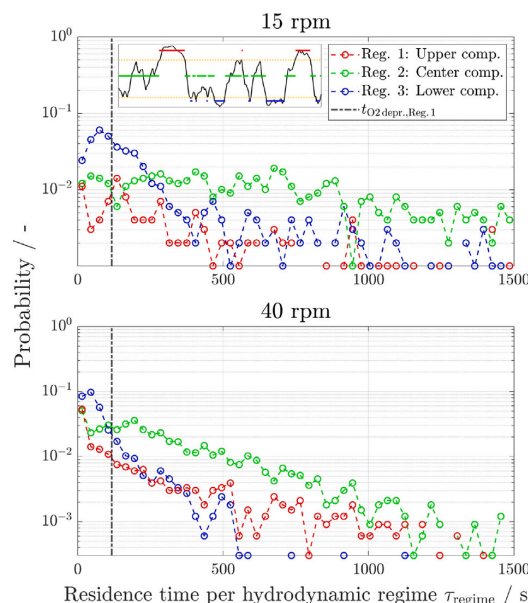


Fig. 9. Based on the calculated hydrodynamic compartment barriers resulting from the circulation time distribution (Fig. 7), residence time probability distributions per regime are shown for the upper (red), center (green) and lower compartment (blue) for both 15 and 40 rpm. The inset depicts the lifeline discretization with the respective color and the vertical dashed line an assumed temporal oxygen deprivation limit for a CHO cell cultivation in the upper compartment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

agitator and the discretization into metabolic regimes with different specific substrate uptake rates (Haringa et al., 2018, 2017). Due to the comparatively large size of the center compartment in this study, for both rpm cases, the LSPs are more likely to stay in the center than in the lower or upper regime. Generally, the RTDs at 15 rpm are more equally distributed than for the 40 rpm case. This means a higher exchange rate for the latter, and thus the higher the impeller frequency the shorter the individual periods of residence times. When analyzing the total regime residence time percentage ratio, it becomes apparent that the LSPs only stay in the upper compartment 11.4 % and 17.6 % of the total recording time ($\approx 264\text{ min}$) in case of 15 and 40 rpm, respectively. The higher value for the latter may be attributed to the slight density shift during the experiments at $T = 37^\circ\text{C}$. It is to be noted that the upper compartment, however, makes up approx. 25 % of the total volume.

Furthermore, with the assumptions that LSPs mimic CHO cells during a cultivation, and that the aeration rate in the process does not influence the inherent measured hydrodynamic behavior, theoretical oxygen deprivation times in the upper compartment can be estimated. Hence, the proportion of cells inside the reactor, which are potentially exposed to oxygen deprivation conditions (Paul et al., 2004) can be ascertained. For a CHO cell cultivation, a maximum oxygen consumption rate is found to be approx. $0.6 \cdot 10^{-16}\text{ mol cell}^{-1}\text{ s}^{-1}$ (Ihling et al., 2022). With a viable cell count of approx. $15 \cdot 10^6\text{ mL}^{-1}$ (De Jesus and Wurm, 2011; Wurm, 2004) and the assumption of a zero-order kinetics, it would take $\approx 117\text{ s}$ (vertical dashed lines in Fig. 9) to reduce the dissolved oxygen from 60 % of air saturation to lower than 10 % at 37°C ($K_{O_2,H_2O} = 93.9\text{ Pa m}^3\text{ mol}^{-1}$, $\xi_{O_2,H_2O}^* = 6.8 \cdot 10^{-3}\text{ kg m}^{-3}$) (Paul et al., 2004). Concluding, 8.9 % and 8.4 % of the entire recording time, oxygen deprivation conditions are likely to occur for 15 and 40 rpm, respectively (see Table 4). A recent scale-down study (Wang et al., 2023) on *Penicillium chrysogenum* demonstrated a significant penicillin productivity reduction during dynamic starvation conditions (150 s)

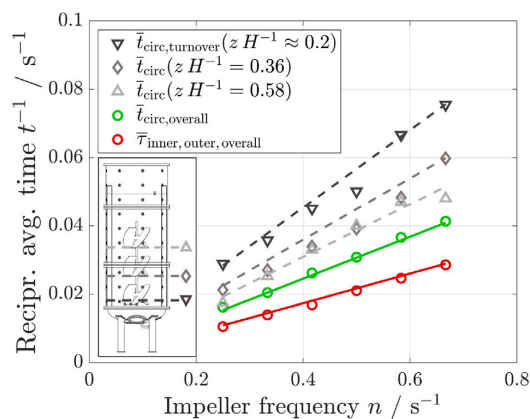


Fig. 10. Reciprocal average times of LSP measurement results (see Eq. (33)) as a function of the impeller frequency. Evaluated times are the average circulation times at three planes in the reactor ($z H^{-1} \in \{ \approx 0.2, 0.36, 0.58 \}$) - black, dark gray and light gray, respectively, the average overall circulation time (green) and the overall added hydraulic residence time (red). The slope k_{circ} reveals the different Flow numbers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by a factor of two. Furthermore, Qian et al. (2014) described dissolved oxygen values of less than 15% as hypoxic conditions for CHO cells, which results in a decrease of protein productivity. Moreover, Gaugler et al. (2023) conducted CHO cell cultivations in a single multi-compartment bioreactor (SMCB) as a scale-down model, mimicking large-scale mixing and found a reduction of the viable cell count in the stationary phase in combination with an increment of lactate concentration while the cells were exposed to enhanced oxygen gradients. Similar results are described by Anane et al. (2021) for dissolved oxygen gradients and by Gao et al. (2016) by means of fibronectin as a biomarker for hypoxic stress in a 5000 L bioreactor.

3.7. Flow number and mixing time correlations

Since most mixing experiments are conducted with six-bladed Rushton turbines (RT-6) and/or three-, four- or six-bladed pitched blade turbines (PBT-3, PBT-4, PBT-6), the latter displays a rough indication for herein calculated Flow number values for the EE impeller agitator, due to their common predominant axial pumping direction. To the best of the author's knowledge, no literature values are available for the EE impeller ABEC design. With the regression method described in Section 2.9, the different Flow numbers are scrutinized. As expected, Fig. 10 indicates an indirect proportionality between the plotted averaged times and the impeller frequency.

With the values shown in Table 3 it becomes apparent that the Flow number based on the average overall circulation time as $N_{Q,c,overall} = 1.00$ seems not to be comparable to literature results, presumably due to the 3-staged design and especially the consideration of the upper compartment, where no active pumping takes place. Taking the ratio between the latter and $N_{Q,r}$, it follows that $\bar{t}_{circ,overall} = 0.71 \cdot \bar{t}_{overall}$, which infers that the LSP's circulation on average covers the axial center region and does not close the loop often enough to cover the bottom and top region sufficiently. This time margin explains again the formation of the previously described compartments with their marked boundaries. In case of a multi-staged agitator, Schubert (2008) suggest that circulation numbers should be referred to one impeller each. Hence, after calculating those for the depicted positions (see Fig. 10), the average Circulation Flow number of $N_{Q,c,1}$, $N_{Q,c,2}$ and $N_{Q,c,3}$ is $\bar{N}_{Q,c} = 1.53 \pm 0.30$, which is in the range of literature results obtained by different LSPs in STRs, although single-staged PBTs with

a lower Po in a reactor with $H d_{STR}^{-1} \leq 1$ were used. Interestingly, $N_{Q,r}$ is very similar to literature values stated as $N_{Q,p}$. And furthermore, Schubert and Liepe's approach of $Q_c \approx Q_p \cdot (d_{STR} d_{imp}^{-1})$ puts this study's results in a fitting ratio, if $N_{Q,p} = N_{Q,r}$ and $d_{STR} d_{imp}^{-1} = 2.19$ so that $\bar{N}_{Q,c} \approx N_{Q,r} \cdot 2.19 = 1.55$ (see Eqs. (30) and (31)).

Those estimations imply that although three impeller stages are installed in this study's reactor resulting in a $H d_{STR}^{-1}$ per impeller stage of 0.7, the overall pumping number is similar to a one-staged EE impeller with a much lower Po number without any compartment formation. Furthermore, the circulation time correlation of Middleton (Middleton, 1979), which is stated as

$$\bar{t}_{circ} = 0.5 \cdot V^{0.30} \cdot \frac{1}{n} \left(\frac{d_{STR}}{d_{imp}} \right)^3 \quad (35)$$

and was obtained with radio pills in multiple reactors ($H d_{STR}^{-1} = 1$) with volumes up to 4.4 m^3 , equipped with one Rushton turbine, underestimates the herein presented results by a factor of 2, albeit the overall added residence time instead of the average overall circulation time. The model described by Schubert and Liepe (Schubert, 2008) slightly overestimates this study's results by $\delta N_{Q,c} \approx 0.38$, yet matches obtained $N_{Q,c,1}$ for the turn-over point. In addition, Herbert et al. (1994) and Nienow (1996) describe the functionality $N_{Q,p} \sim Po^{1/3}$, which would yield $N_{Q,p} \in \{0.91, 1.06\}$ for $Po \in \{3.35, 5.3\}$ for a single-staged EE in a 3 L STR (not shown here, equal reactor ratios) and the three-staged EE in this study, respectively. Those values are slightly higher than those described by Simmons et al. (2007) and Zhu et al. (2009), who use, however, the Applikon design with a lower Power number. Moreover, the Exchange Flow numbers provide model parameters regarding the root mean square of the exchange flow rate at both the lower compartment boundary (at approx. $z H^{-1} = 0.15$) and the upper compartment boundary (at approx. $z H^{-1} = 0.75$). Results show in this single-phase flow double the exchange flow rate towards the lower regime than towards the upper regime.

In Fig. 11 (left) the results in terms of the relation between the average overall circulation times (green dots), average overall added residence times (red dots) and global mixing times (blue dots) against the volumetric power input are depicted. It can be observed that all three plotted times show a general asymptotic decreasing trend towards a respective characteristic value with an increasing $P V^{-1}$ ratio. Moreover, the global mixing time trend is congruent with the overall added residence times within their standard deviation or uncertainty. This is especially the case for higher specific power inputs. In case of lower volumetric power inputs, the mixing times are similar to those of the average overall circulation time within their standard deviation or uncertainty. The low standard deviations of the latter can be explained by taking the weighted average circulation time value at the reactor heights and considering their total number of counts.

Similar to the results in Bisgaard et al. (2021a), in Fig. 11 (right) a linear regression of both the average overall circulation time and the overall added residence time to the global mixing time is denoted. With a slope of 1.00 ± 0.29 and 0.64 ± 0.20 , they show a comparatively high confidence bound for their fit, respectively. Especially the latter lies within a 15% margin of the interpolated mixing times, however, they both could also be described by a sigmoidal trend. In contrast to literature statements, which indicate $3 \cdot t_{circ} < t_{mix,global,95} < 5 \cdot t_{circ}$ (Nienow, 1996, 1998; Voncken et al., 1964; Khang and Levenspiel, 1976) based on conductivity measurements, however, with utilizing a single-staged RT-6 setup, Bisgaard et al. (2021a) found a relation of $t_{mix,global,95} = 2.6 \cdot t_{circ}$ with a single-staged PBT-4 and $t_{mix,global,95} = 2.2 \cdot t_{circ}$ with a single-staged RT-6 utilizing LSPs. They interpret their low values with the tracer detection position described in Holmes et al. (1964) at half of the liquid height, which therefore represents only "half a circulation" (Bisgaard et al., 2021a). This does not explain, however, the minor difference of only 0.4 between the RT-6 and PBT-4 impeller, although both clearly exhibit a different hydrodynamic flow pattern. Further comparing their

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Table 3

Turn-over constants k_{to} according to Eq. (33), and Flow numbers according to Eqs. (30) and (34) (Pumping number $N_{Q,p}$, Circulation Flow number $N_{Q,c}$, Residence Flow number $N_{Q,r}$ and Exchange Flow number $N_{Q,e}$) with literature values and correlations. Respective slopes are depicted in Fig. 10. h_{bm} : off-bottom clearance to first impeller stage; c_1 : momentum flow number; rms: root mean square.

$k_{to}/-$	Flow number/-	Comments
–	$N_{Q,p} \sim Po^{1/3} \in \{0.91, 1.06\}$	Pitched Blade/Hydrofoils, with $Po \in \{3.35, 5.3\}$ (Nienow, 1996; Jaworski et al., 1996; Weetman and Oldshue, 1988)
–	$N_{Q,p} = 0.87$	1x up-pumping EE (Applikon), $Po \approx 1.7$, $d_{imp} d_{STR}^{-1} = 0.45$ (Simmons et al., 2007)
–	$N_{Q,p} = 0.73$	1x down-pumping EE (Applikon), $Po \approx 2.1$, $d_{imp} d_{STR}^{-1} = 0.45$ (Zhu et al., 2009)
–	$N_{Q,c} = 1.91$	Axial flow, $h_{bm} = 0.65$ m, $c_1 = 0.9$ (Schubert, 2008) (p. 1076)
0.13	$N_{Q,c} = 1.45$	LSP meas.: 1x down-pumping PBT-3, $Po \approx 1.2$, $d_{imp} d_{STR}^{-1} = 0.3$ (Reinecke, 2013)
–	$N_{Q,c} = 1.39$	LSP meas.: 1x down-pumping PBT-4, $d_{imp} d_{STR}^{-1} = 0.32$ (Bisgaard et al., 2021a)
0.06	$N_{Q,e,overall} = 1.00$	this study, avg. overall circulation time
0.11	$N_{Q,e,1} = 1.85$	this study, avg. circulation time at $z H^{-1} \approx 0.2$ (turn-over point)
0.09	$N_{Q,e,2} = 1.47$	this study, avg. circulation time at $z H^{-1} = 0.36$
0.08	$N_{Q,e,3} = 1.26$	this study, avg. circulation time at $z H^{-1} = 0.58$
0.04	$N_{Q,r} = 0.71$	this study, overall added inner and outer residence times
–	$N_{Q,e,low} = 1.31 \pm 0.07$	this study, rms of inner and outer volume flow at $z H^{-1} = 0.15$
–	$N_{Q,e,up} = 0.62 \pm 0.08$	this study, rms of inner and outer volume flow at $z H^{-1} = 0.75$

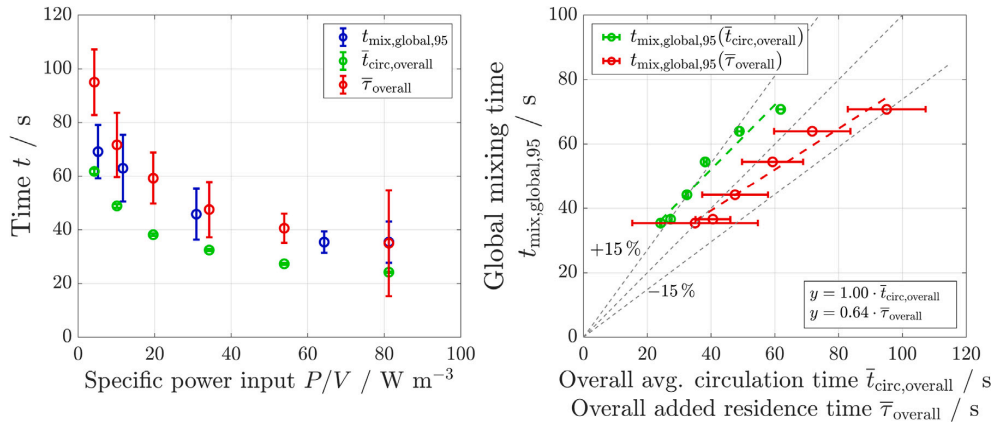


Fig. 11. Measured global mixing time (blue dots), average overall circulation time (green dots) and overall added hydraulic residence time (red dots) as a function of the specific power input (left) and the last two as a function of extrapolated global mixing times according to measured values with a respective linear regression (right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

results to this study's results quantitatively, concludes to the possibility that either their pH probe measured excessive mixing times due to a long response time or low circulation times due to an insufficient flow-following behavior in a comparatively small tank and a similar LSP size. Moreover, a model approach by van de Vusse (1962) considering up to four mixing stages assumes a complete homogenization of the tank after about one circulation, which would fit this study's result. Nevertheless, all contemplate a one-staged agitator with a $H d_{STR}^{-1} = 1$ ratio.

Additionally, for a comparison in a dimensionless way, obtained times can be multiplied with respective impeller frequencies and plotted as a function of the stirrer Reynolds number (Zlokarnik, 2001; Rosseburg et al., 2018), as seen in Fig. 12.

Correlating those to literature results should be treated with caution due to different $H d_{STR}^{-1}$ ratios (mostly = 1), single-staged agitators and especially mostly utilized RT-6 or PBT impellers on much smaller scales. Relevant mixing time correlations, which include the Circulation Flow number (Nienow, 1998) ($H d_{STR}^{-1} = 1$, RT-6, assumption that system is homogenized after five circulations),

$$t_{mix,Nie} \cdot n = 3.9 \cdot \left(\frac{d_{imp}}{d_{STR}} \right)^{-3} \cdot N_{Q,c}, \quad (36)$$

or the number of impellers i_{imp} (Vasconcelos et al., 1995) (1x RT-6 per $H d_{STR}^{-1} = 1$),

$$t_{mix,Vas} \cdot n = 2.3 \cdot \exp \left(0.68 \cdot \frac{d_{STR}}{d_{imp}} + 0.83 \cdot i_{imp} \right), \quad (37)$$

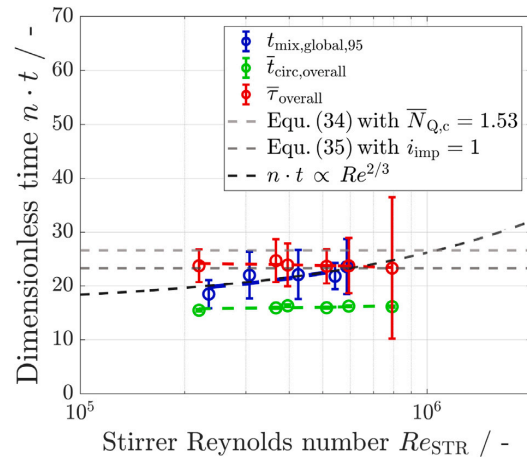


Fig. 12. Dimensionless global mixing time (blue dots), average overall circulation time (green dots) and overall added hydraulic residence time in dependency of the stirrer Reynolds number. Comparison between literature correlations and experimental results. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4

Overview of results of different St numbers, average overall circulation times, overall added residence times, respective correlated global mixing times, and percentages of theoretical O_2 deprivation conditions in the upper compartment.

	Stirring frequency n/rpm					
	15	20	25	30	35	40
St in DW (based on resp. Taylor scale L_E)/–	4.0	4.7	5.1	5.4	5.7	6.2
St in IS (based on resp. Taylor scale L_E)/–	4.5	5.3	5.9	6.2	6.6	7.2
St (meso-scale)/–	0.17 < St < 0.18 ≈ 0.004					
St (macro-scale)/–						
$\bar{t}_{\text{circ,overall}}/\text{s}$	61.8	48.9	38.2	32.4	27.3	24.2
$\Delta \bar{t}_{\text{circ,overall}}/\text{s}$	0.9	0.7	0.4	0.4	0.3	0.2
$\bar{t}_{\text{overall}}/\text{s}$	95.1	71.7	59.3	47.5	40.6	35.0
$\Delta \bar{t}_{\text{overall}}/\text{s}$	12.2	12.0	9.6	10.3	5.5	19.7
Correlated $t_{\text{mix,global,95}}/\text{s}$	70.8	64.0	54.5	44.2	36.6	35.4
Percentage of time in the upper compartment under theoretical O_2 deprivation conditions/%	8.9	9.3	8.3	11.3	6.9	8.4

anticipate a constant value when operating within the turbulent regime $Re_{\text{STR}} > 10^4$ and are overestimated by an approximate factor of 2 (if $N_{Q,c} = 1.0$) and 5.3 (if $i_{\text{impeller}} = 3$). Interestingly, the first shows a reasonable value if $\bar{N}_{Q,c} = 1.53$ is used ($t_{\text{mix,Nie}} = 26.6$ s) and the second, if $i_{\text{imp}} = 1$ ($t_{\text{mix,Vas}} = 23.3$ s), albeit herein measured results are clearly increasing with an increasing stirrer Reynolds number as already demonstrated in a previous study by Rosseburg et al. (2018). Generally, the trend of the global mixing time follows $n \cdot t \propto Re^{2/3}$, which is very similar as described by Zlokarnik ($n \cdot t \propto Re^{0.7}$) for a single-staged propeller if $Re_{\text{STR}} > 10^4$ (Zlokarnik, 1967). The correlation provided by Khang and Levenspiel (Khang and Levenspiel, 1976) by means of the decay rate constant K for propeller mixers (if $Re \geq 10^4$) is found to be $(n K^{-1}) (d_{\text{imp}} d_{\text{STR}}^{-1})^2 = 8.9 \pm 5.5$, which is overestimated by a factor of 10 and proportional to the increase of Po . As reported by Holmes et al. (1964), in terms of both the average overall circulation time and overall added residence time, they approach constant values, which are in this study 15.5 ± 0.8 and 24.5 ± 1.4 , respectively. In light of utilizing a three-staged axially pumping EE impeller agitator with a $H d_{\text{STR}}^{-1} = 2.1$, their plotting approach could be extended so that $n \cdot \bar{t}_{\text{circ,overall}} \cdot (d_{\text{imp}} d_{\text{STR}}^{-1})^2 \cdot d_{\text{STR}} \cdot (H n_{\text{imp}})^{-1}$, which would yield 0.51 and being approximately half of the value achieved by Holmes et al. with a single-staged RT-6 and a $H d_{\text{STR}}^{-1} = 1$ (Vrábel et al., 2000; Sieblitz et al., 2011).

4. Conclusion

In this study, Lagrangian Sensor Particles (LSPs) with a macro-scale Stokes number $St \approx 0.004$, equipped with a pressure sensor, are utilized to investigate lifelines of cells in a 15,000 L stirred tank reactor (STR) with three Elephant Ear (EE) impellers. To hydrodynamically characterize the entire STR and analyze single-phase mixing heterogeneities within the bioreactor in form of compartments, the vertical probability of presence of the LSPs, axial velocity profiles, axial circulation time distributions and residence time distributions are quantified. The culmination of this is a Lagrangian regime analysis. Results are compared to experimentally determined global mixing times for the same setup and literature results from similar bioreactors of different scales.

For the examined operating points, the vertical probability of presence is similarly distributed throughout the vertical reactor center, however, decreases in the top and bottom of the STR. Solely in the case of the operating point with $n = 15$ rpm, the LSPs show a higher probability of presence towards the bottom of the reactor, emphasizing the sensitivity of the LSP to fluctuating densities. The characteristic down-pumping behavior of the EE impellers is depicted by the axial velocity distributions for both downwards and upwards flow. Peaks are evident for the upper impeller stage regarding the downwards flow (suction zone) and at the lowest impeller stage regarding the upwards flow (redirection zone).

By investigating the axial circulation time distributions (CTDs) over the reactor height, defined as the time between to consecutive LSP passages over a certain axial height, the resulting profile reveals distinctively shorter circulation times in the axial center region than in the regions below and above. In the last two, comparatively high uncertainties lead to the conclusion that a 10-fold longer measurement time is necessary to show a significant statistic for CTDs in the reactor bottom and top region. Nonetheless, for all operating points, three compartments are discerned, with an average overall circulation time being calculated.

With the assumption of a predominantly axial flow profile, the hydraulic residence time distribution (RTD) over the reactor height is calculated by introducing a model based on gained axial velocities. Inner and outer residence times reveal a similar profile as the previously derived CTDs with three compartments.

Consequently, a Lagrangian regime analysis is conducted based on the compartmentalization of the CTD data with a discretization of the LSP lifelines. These single-phase flow results allude to a potential oxygen deprivation zone for CHO cells in the upper compartment of at least 7 % of the experimental time, if similar hydrodynamics during a fermentation are assumed.

Furthermore, under the consideration of previous results, different Flow number calculations demonstrate that LSPs can be utilized to characterize impeller systems, and provide model parameters. Although literature values for a three-staged EE impeller system on industrial scale are not available, presented results are comparable.

Contrary to literature stating that the ratio of the global mixing time to circulation time falls between 2.2 and 5.0, this study, admittedly using a different impeller setup, reveals that both values exhibit no such dependency and instead maintain a ratio of 1.0. With the exception for $n = 15$ rpm, overall added residence time values are similar to the global mixing time, while within the respective uncertainty or standard deviation. Results highlight, that all three presented methods describe mixing on a macro-scale, for which the LSPs show a sufficiently small Stokes number.

This study demonstrates that LSP technology serves as an effective tool for investigating large-scale stirred tank reactors. Results showcase the capability of an LSP to detect hydrodynamic compartments, while not requiring optical access. To comprehensively determine the fluid flow, it is essential to further advance the LSPs through sensors making their localization possible, as they are currently limited to axial resolution. To better represent a cell within a bioreactor through an LSP and discern the respective process conditions experienced by a cell over the course of its lifeline, sensors for process parameters such as temperature, pH, dissolved oxygen and conductivity will be installed in the near future. Moreover, further development is necessary regarding their balancing, meaning the influence the position of the respective center of mass has on an LSP for more accurate results in detecting meso-scales and vortices. For smaller reactor scales, miniaturization proves highly advantageous, however, as this study shows, in

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order to determine compartments on a macro-scale the flow-following capability of available LSPs proves to be sufficient.

CRedit authorship contribution statement

Sebastian Hofmann: Conceptualization, Methodology, Project administration, Software, Writing – original draft, Writing – review & editing, Supervision, Validation, Visualization, Data curation, Formal analysis, Investigation. **Lukas Buntkiel:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ryan Rautenbach:** Validation, Writing – review & editing, Conceptualization, Formal analysis, Investigation. **Lena Gaugler:** Conceptualization, Formal analysis, Investigation, Validation, Writing – original draft. **Yifan Ma:** Software, Conceptualization, Methodology, Resources. **Ingrid Haase:** Investigation, Methodology, Conceptualization, Data curation. **Jürgen Fitschen:** Investigation, Methodology, Data curation. **Thomas Wucherpfennig:** Funding acquisition, Resources, Supervision. **Sebastian Felix Reinecke:** Funding acquisition, Methodology, Supervision. **Marko Hoffmann:** Funding acquisition, Project administration, Resources, Writing – review & editing. **Ralf Takors:** Funding acquisition, Project administration, Resources, Supervision. **Uwe Hampel:** Funding acquisition, Resources, Supervision. **Michael Schlüter:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

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.

Data and code availability

Utilized MATLAB® scripts that were developed in order to evaluate the data can be publicly accessed at our [Gitlab project](#). Raw data and results are publicly accessible in our [DaRUS repository](#). Please contact Sebastian Hofmann or Lukas Buntkiel for further information.

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Appendix A. Exemplary axial movement of an LSP

See Fig. 13.

Appendix B. Axial probability of presence at 35 rpm

See Fig. 14.

Appendix C. Axial velocity distributions at 35 rpm

See Fig. 15.

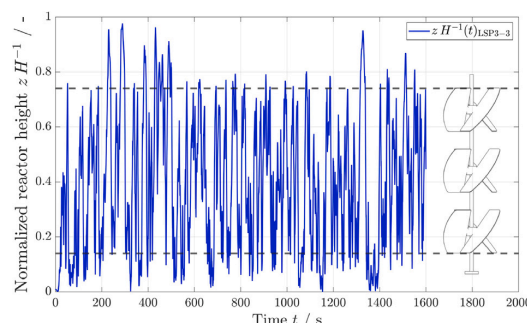


Fig. 13. Exemplary axial movement of the LSP (ID 3) of trial no. 3 (LSP3-3) at an impeller frequency of $n=35$ rpm over time with indicated position of the Elephant Ear (EE) impellers and respective upper and lower edge (gray dashed lines).

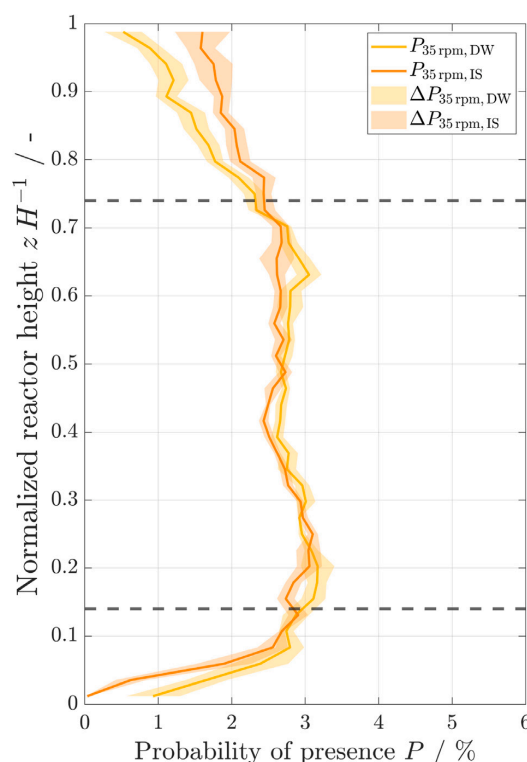


Fig. 14. Axial probability of presence over the normalized reactor height ($H=4.2$ m) for four LSP data sets for each trend at 35 rpm in DW at $T=20$ °C (yellow line) and in IS at $T=37$ °C (orange line) for a vertical bin size of 10 cm. Shaded areas show uncertainty Δ of respective trend. Dashed lines indicate the lower and upper edge of EE impellers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

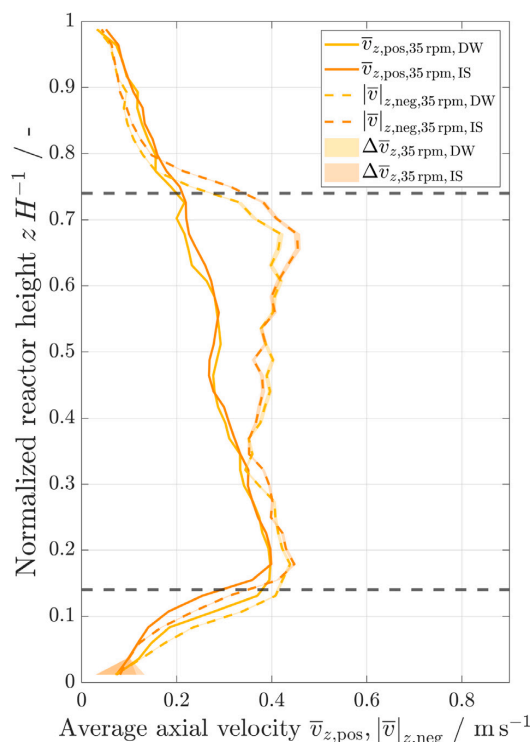


Fig. 15. Average positive (solid lines, upwards flow) and negative (dashed lines, downwards flow) axial velocity distributions over the normalized reactor height ($H = 4.2$ m) for four LSP data sets for each trend at 35 rpm in DW at $T = 20^\circ\text{C}$ (yellow line) and in IS at $T = 37^\circ\text{C}$ (orange line) for a vertical bin size of 10 cm. Shaded areas show uncertainty Δ of respectively colored data set. Dashed lines indicate the lower and upper edge of EE impellers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Chapter 6

Lagrangian Sensor Particles for detecting hydrodynamic heterogeneities in industrial bioreactors: experimental analysis and Lattice-Boltzmann simulations

This chapter reproduces the publication: Hofmann, S., Rautenbach, R., Buntkiel, L., Brouwers, I.S., Gaugler, L., Barczyk, J., Fitschen, J., Reinecke, S., Hoffmann, M., Takors, R., Hampel, U., Schlüter, M., 2025. Lagrangian Sensor Particles for detecting hydrodynamic heterogeneities in industrial bioreactors: Experimental analysis and Lattice-Boltzmann simulations. Chemical Engineering Journal Advances 22, 100744 [Hof25f]. Utilized data and mathematical analyses can be accessed at the DaRUS repository [Hof25a] or in the Gitlab repository [Hof25d].

Lifelines on industrial scale and in Lattice-Boltzmann simulations

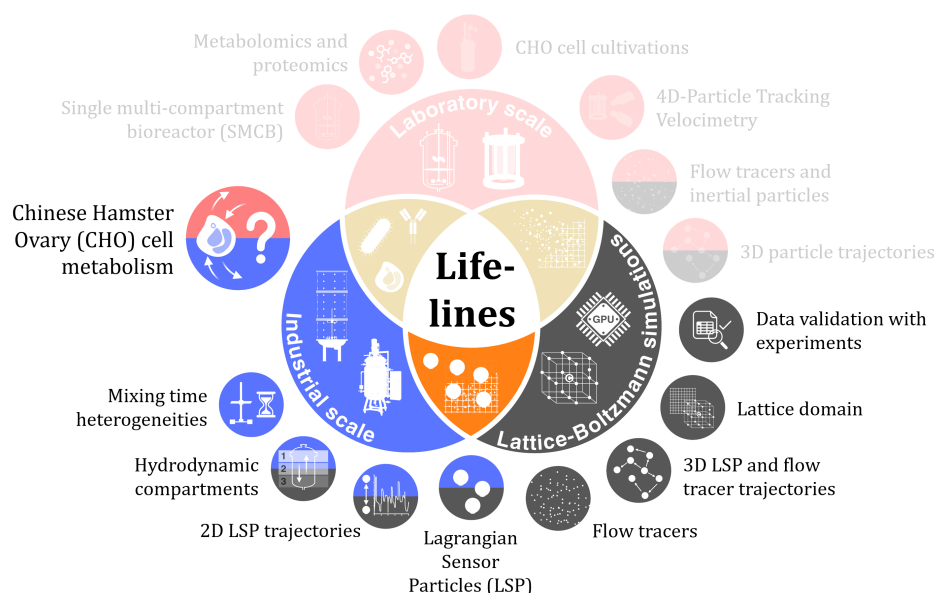


Figure 6.1: Figurative representation of the two investigation frameworks of the third publication.

As demonstrated in the second publication, despite solely being equipped with a pressure sensor and thus being limited to an axial resolution, and having a relatively large size of $d_{LSP} = 40\text{ mm}$, state-of-the-art Lagrangian Sensor Particles (LSP) are capable of delivering valuable insights into industrial-scale bioreactors. Experimental trajectories enabled the quantification of axial velocity distributions, circulation and residence time distributions, and even revealed three hydrodynamic compartments, which could be utilized for a Lagrangian regime analysis. These findings not only aid in identifying macro-scale heterogeneities but also offer parameters for scale-down models. Additionally, for the first time, flow numbers and model parameters were calculated for a three-staged Elephant Ear impeller system, further showcasing the LSP's potential in fluid dynamic bioreactor characterization. However, limitations became apparent: due to the lack of time-resolved 3D particle positions and an undersized number of data points, only meso- and macro-scale Stokes numbers could be estimated. Moreover, the uniqueness of the impeller configuration reduced comparability with literature datasets.

To address these limitations, the third publication introduces additionally complementary Lattice-Boltzmann simulations (Fig. 6.1). Here, experimental LSP data from a bioreactor equipped with a widely used Rushton turbine and pitched blade turbine setup are bench-

marked against numerically resolved LSPs and massless flow tracers. These simulations allow access to fully resolved 3D flow fields and spatially distributed Stokes numbers, providing a deeper insight into particle flow interactions. Furthermore, the estimated necessary recording time from the second publication is applied in the third publication. Once again, three hydrodynamic compartments are identified, now validated by experimental local mixing time distributions. While the simulations show strong agreement with experimental results on several metrics, discrepancies highlight the importance of experimental validation, particularly with regard to the axial probability of presence. Minor differences in densities, the utilized particle-to-grid size ratio, an inconsistent mass distribution, limited ergodicity in terms of particle numbers and recording times, wall clock times ranging from five to seven days, the simulations underscore that numerical approaches cannot fully replace experiments, yet are critical for understanding LSP study results. Nevertheless, although it is once again calculated that LSP sizes of approx. 1 mm would benefit to characterize the bioreactor on micro-scale, this combined analysis confirms that LSPs are capable of capturing essential flow structures on meso- and macro-scale, confirming their use as a practical tool for a fluid dynamic characterization of industrial bioreactors and supporting the design of reliable scale-down models.

Lagrangian Sensor Particles for detecting hydrodynamic heterogeneities in industrial bioreactors: experimental analysis and Lattice-Boltzmann simulations

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Lagrangian Sensor Particles for detecting hydrodynamic heterogeneities in industrial bioreactors: Experimental analysis and Lattice-Boltzmann simulations

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ABSTRACT

This study analyzes trajectories of three particle types in an industrial-scale bioreactor, equipped with a Rushton turbine and a pitched blade turbine, to characterize hydrodynamic compartments. The trajectories obtained from measurements with Lagrangian Sensor Particles (*LSP,exp*) are compared to those generated by Lattice-Boltzmann large eddy simulations (LB LES). The latter method is used to reproduce analogous simulated LSPs (*LSP,sim*) as resolved particles. Additionally, for benchmarking purposes, massless tracer particles (*tracer,sim*) are incorporated to accurately represent fluid flow dynamics. Discrepancies in the axial probability of presence and velocity between *LSP,exp* and *LSP,sim* likely stem from differences in mass distribution, density, number of particles, and ratio of particle size to grid. A necessarily high *LSP,sim* volume fraction in LB LES leads to increased collisions and clustering, negatively impacting flow dynamics, and reducing turbulent kinetic energy by at least 3%. Circulation and residence time distributions for the three types of particles identify three hydrodynamic compartments within the bioreactor, validated by local mixing time distributions. The ratio of overall average circulation time to global mixing time is $\Theta_{\text{glob},95} \approx 3.0 \cdot \bar{t}_{\text{circ}}$ for *LSP,exp*, which largely corresponds to literature results. A theoretical LSP size of $d_{\text{p,th}} \approx 1 \text{ mm}$ is estimated to be flow following on micro-scale in the bulk phase, if a Stokes number of $St = 0.1$ is assumed. However, Stokes number estimations confirm that *LSP,exp* are capable to follow flow patterns on the meso-scale and macro-scale with $St \approx 0.2$ and $St \approx 0.002$, respectively. Hence, hydrodynamic structures at length scales greater than or equal to the size of the impeller can be investigated by current state-of-the-art LSPs, which proves their technological readiness for industrial bioreactors.

1. Introduction

For industrial biopharmaceutical production, stirred tank reactors (STR) are essential. The characterization and process monitoring of such reactors is generally limited to traditional Eulerian wall-mounted probes, which measure process conditions such as temperature, pH, conductivity, oxygen, and many more. However, the downside of such probes is that they are locally fixed and do not represent the entirety of the reactor volume.

On an industrial scale, heterogeneities in the form of compartments [1] occur and have adverse effects on the dynamics of the cell

cycle and energy levels when exposed to strong fluctuations in the media during mixing [2–4]. Mixing times, ranging from approximately ten seconds and longer, exceed the duration of key intracellular processes and, consequently, may lead to population heterogeneity. The extent depends on the severity and time scale to which the cell is exposed to gradients in repeatedly unpredictable microenvironments [5–7]. This issue becomes even more complex when existing production processes are intensified using perfusion systems [8], or when novel bioprocesses are developed with artificial microbial consortia [9–12]. From

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Nomenclature			
Arabic symbols			
$\dot{V}_j$	Volume flow rate in reactor slice j / $\text{m}^3 \text{s}^{-1}$	p_0	Ambient pressure / Pa
$\bar{t}_{\text{circ,overall}}$	Overall average circulation time / s	p_{res}	Pressure resolution / Pa
$\bar{d}_{\text{travel}}$	Maximum traveled distances / m	PCP	Particle collision or perturbation probability / %
c_i	Finite velocity in discrete lattice D3Q19 / m s^{-1}	Po	Power number / –
F_{ext}	External forces / N	r_{imp}	Impeller radius / m
u'	Fluid velocity fluctuation / m s^{-1}	r_{shift}	Center of mass offset / mm
$u'_{\text{rms,sim}}$	Root mean square of simulated fluid velocity fluctuations / m s^{-1}	Re_{STR}	Stirrer Reynolds number / –
u_{stoch}	Stochastic fluid velocity fluctuation magnitude / m s^{-1}	Re_p	Particle Reynolds number / –
v'	Particle velocity fluctuation / m s^{-1}	St	Stokes number / –
$V_{\text{slip,stoch}}$	Stochastic slip velocity / m s^{-1}	St_{MF}	Stokes number of the mean flow / –
A_j	Surface area of reactor slice j / m^2	St_{stoch}	Stochastic Stokes number / –
B	Fitting constant / –	T	Temperature / $^{\circ}\text{C}$
b	Baffle width / m	t_{circ}	Circulation time / s
C	Impeller off-bottom clearance / m	$t_{\text{rec,single,exp}}$	Recording time of single experimental LSP / s
c_D	Drag coefficient / –	$t_{\text{sim,max}}$	Maximum physical simulated time per particle / s
C_s	Smagorinsky coefficient / –	$t_{\text{rec,tot,exp}}$	Total recording time of experimental LSPs per rpm case / h
Co	Courant number / –	$t_{\text{rec,tot,sim}}$	Total recording time of simulated particles per rpm case / h
d_{imp}	Impeller diameter / m	u	Velocity of the fluid / m s^{-1}
d_{LSP}	LSP diameter / m	u_{tip}	Impeller tip speed / m s^{-1}
$d_{\text{p,th,MF}}$	Theoretical maximum particle diameter in mean flow / m	V	Reactor working volume / m^3
$d_{\text{p,th,stoch}}$	Theoretical maximum particle diameter, stochastically / m	v	Velocity of the LSP / m s^{-1}
d_p	Particle diameter / m	$v_{z,\text{max}}$	Maximum axial velocity / m s^{-1}
d_{STR}	Reactor diameter / m	v_z	Axial velocity / m s^{-1}
f	Particle distribution function / $\text{m}^{-6} \text{s}^3$	w	Weight for average value / –
f^{eq}	Equilibrium particle distribution density / $\text{m}^{-6} \text{s}^3$	w_G^0	Superficial gas velocity / mm s^{-1}
H	Reactor filling height / m	z	Specific height in reactor / m
h	Impeller spacing / m	$z H^{-1}$	Normalized reactor height / –
h_j	Height of reactor slice j / m	Greek symbols	
I'_t	Normalized gray scale intensity over time / –	Δ	Uncertainty
k	Turbulent kinetic energy / $\text{m}^2 \text{s}^{-2}$	Δx	Lattice spacing in numerical simulations / m
L	Characteristic fluid flow length scale / m	η	Dynamic viscosity / Pa s
L_E	Taylor scale / m	γ	Linear correlation coefficient / –
L_{MF}	Flow length scale of the mean flow / m	Λ	Turbulence length scale / m
M	Total trials per rpm case / –	λ_K	Kolmogorov length scale / m
m_{LSP}	LSP mass / kg	$\lambda_{K,\phi}$	Min. Kolmogorov length scale / m
n	Impeller frequency / rpm	$\mathcal{O}$	Magnitude / –
N_{imp}	Number of impellers / –	ν	Kinematic viscosity / $\text{m}^2 \text{s}^{-1}$
$N_{\text{LSP,sim}}$	Number of simulated LSPs in reactor / –	Ω	Collision parameter / $\text{m}^{-6} \text{s}^2$
N_j	Number of counts in bin j / –	$\bar{\tau}_{\text{overall}}$	Overall cumulative residence time / s
P	Global power input / W	ϕ	Local energy dissipation ratio / –
P	Probability / –	$\Phi_{\text{LSP,exp}}$	Experimental LSP volume fraction / –
p	Hydrostatic pressure / Pa	$\Phi_{\text{LSP,sim}}$	Experimental LSP volume fraction / –
$P V^{-1}$	Global power per volume input / W m^{-3}	ρ	Density / kg m^{-3}
		ρ_f	Fluid density / kg m^{-3}
		ρ_p	Particle density / kg m^{-3}
		σ^2	Intensity variance / –
		τ	Residence time / s
		τ_c	Collision time / s

a hydrodynamic point of view, the entire reactor shows a substantial variation of energy dissipation rates and resulting Kolmogorov length scales, which may additionally decrease the quality and quantity of the product [13,14].

In this case, numerical simulations support the identification of metabolic regimes in the STR and are able to perform a lifeline analysis for occurring mass transfer limitations [15–17]. Eventually, these

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τ_{regime}	Residence time per hydrodynamic regime / s
τ_f	Characteristic time of the flow field / s
τ_p	Particle response time / s
θ^*	Relative local mixing time / s
$\theta_{\text{glob},95}$	Global mixing time / s
$\theta_{\text{loc},95}$	Local mixing time / s
$\varepsilon_{T,\text{loc}}$	Local energy dissipation rate / W kg^{-1}
ε_T	Energy dissipation rate / W kg^{-1}
ζ	Continuous velocity of particles / m s^{-1}
Abbreviations	
4D-PTV	4D-Particle Tracking Velocimetry
BTB	Bromothymol blue
CHO	Chinese Hamster Ovary
CTD	Circulation time distribution
D3Q19	Lattice-Boltzmann lattice structure
DW	Deionized water
exp	Experimental
GPU	Graphical processor unit
HCl	Hydrochloric acid
HZDR	Helmholtz-Zentrum Dresden-Rossendorf
IMU	Inertial Measurement Unit
IPO	Injection point
LB LES	Lattice-Boltzmann large eddy simulations
LED	Light Emitting Diode
LP	Lagrangian Particle
LSP,exp	Experimental LSPs
LSP,sim	Simulated LSPs
LSP	Lagrangian Sensor Particle
LX 500	Lattice points across reactor diameter
mag	Magnitude
MF	Mean flow
NaCl	Sodium chloride
NaOH	Sodium hydroxide
PBT-6	6-bladed Pitched blade turbine
PDF	Probability density function
rms	Root mean square
RT-6	6-bladed Rushton turbine
RTD	Residence time distribution
sim	Simulated
stoch	Stochastic
STR	Stirred Tank Reactor
TKE	Turbulent kinetic energy
tracer,sim	Simulated tracer particles

combined analyzes are utilized for modeling scale-down bioreactors to understand large-scale phenomena on a laboratory scale, improve process parameters, and predict product outcomes when scaling back up. It enables the investigation of cell-bioreactor interactions with high flexibility through various concepts and stress responses [18–21]. However, numerical simulations have a limitation in accurately predicting realistic bubble size distributions for dense bubbly flows in industrial-sized bioreactors. Applying highly resolved simulations to implement bubble breakup [22] and coalescence [23] is very demanding due to the high computational cost [24]. Hence, the cell's metabolic pathway in multiphase flows and subsequent data for scale-down models lack accuracy, since oxygen stress conditions lead to a decrease in production rate [25,26].

This is the reason why experiments on heterogeneities, in particular on an industrial scale, are crucial to deliver further validation data. Therefore, various research groups have developed and successfully implemented Lagrangian Sensor Particles (LSP), which dynamically measure conditions within the STR, while not being locally fixed to the reactor wall [27–29]. The idea is that such an LSP follows the fluid flow in its characteristic way, essentially mimicking the lifetime [2,30,31] of a microorganism during cultivation and providing the desired parameter readings. Recent publications discuss their flow-following capability and its ability to highlight hydrodynamic heterogeneities in an industrial-scale bioreactor [32–34]. However, to what extent such LSPs are flow-following and represent lifelines of a cell deserves to be studied more in detail. Moreover, determining its location using an inertial measurement unit (IMU), and thus achieving a full spatiotemporal resolution over a long period of time, is challenging [29,35,36].

In turn, Lattice-Boltzmann large eddy simulations (LB LES) demonstrate a state-of-the-art methodology for obtaining detailed fluid flow information and transient flow structures in an STR [37–39], and even largely predict the trajectories of small inertial particles in a single-phase system [34,40]. Yet, for the latter, careful consideration must be undertaken, as the particle diameter should not exceed the respective lattice spacing [41]. As soon as the particles reach the size of an LSP and thus are much larger than the lattice spacing, the *resolved discrete element particle* model enhances the capabilities of inertial particles by explicitly resolving the flow field in the boundary layer around the particle and computing associated forces [42]. Eventually, the numerical simulation of LSPs would yield the desired fully resolved spatiotemporal resolution, however, it lacks information on the quality of the trajectories.

To this end, this study compares three particle types: trajectories obtained from measurements with LSPs with replicas from a resolved particle Lattice-Boltzmann large eddy simulation (LB LES) and also massless tracers depicting Chinese Hamster Ovary (CHO) cells in a 15,000 L bioreactor. By doing so, the reactor, which is equipped with a Rushton turbine and a pitched blade turbine, is hydrodynamically characterized, whereupon the compartments and mixing heterogeneities are identified by data gathered via LSPs and validated by experimental mixing time trials. Eventually, a Lagrangian regime analysis and Stokes number estimations provide crucial information about potential oxygen deprivation zones and technological improvements of the reactor system. The ultimate goal of the study is to demonstrate the technological readiness of LSPs and further utilize the information gained to advance the development of scale-down models.

2. Materials and methods

2.1. 15,000 L stirred tank reactor and impellers

The stirred tank reactor (STR) utilized is a replica of an industrial bioreactor, made of acrylic glass, and has been erected in collaboration with Boehringer Ingelheim Pharma GmbH & Co. KG [43]. It is utilized for both experimental investigations and Lattice-Boltzmann large eddy simulations (LB LES). With its inner diameter of $d_{\text{imp}} = 2.0 \text{ m}$, a dished head at the reactor bottom, and a filling level of $H = 4.27 \text{ m}$, the total working volume in this study is $V = 12,500 \text{ L}$ (Fig. 1). It is filled with deionized water (DW) at a temperature of $T = (22.0 \pm 0.3)^\circ\text{C}$ at an estimated viscosity of $\eta = 0.95 \cdot 10^{-3} \text{ Pa s}$ and measured densities as described in Table 1 (Anton Paar DMA 35). The STR is equipped with three equally spaced baffles of width $b = 0.1 \cdot d_{\text{STR}}$ and a bottom-mounted magnetic agitator (ZETA BMRF XXL) with two impeller stages, namely a six-bladed Rushton turbine (RT-6, first stage) and a six-bladed pitched blade turbine (PBT-6, second stage) with a diameter of $d_{\text{imp}} = 0.665 \text{ m}$, which corresponds to a ratio of $d_{\text{imp}} \cdot d_{\text{STR}}^{-1} = 0.33$. The off-bottom clearance is $C = 0.3 \cdot d_{\text{STR}}$ and the impellers are spaced at $h \cdot d_{\text{imp}}^{-1} = 1.65$. Impeller geometries are also described in Šrom et al. [13].

Table 1

Overview of experimental parameters for deionized water (DW) and Lagrangian Sensor Particles (LSPs).

	Deionized water (DW)
Temperature T / °C	(22 ± 0.3)
Dynamic viscosity η ($\cdot 10^{-3}$) / Pa s	0.95
Fluid density ρ_f / kg m $^{-3}$	(998.1 ± 0.4)
LSP density ρ_p / kg m $^{-3}$	$\rho_f \leq \rho_p \leq (\rho_f + 1.0)$

This study evaluates four impeller frequencies n between 32 rpm and 63 rpm (Table 2), which correspond to the respective stirrer Reynolds numbers calculated as

$$Re_{STR} = \frac{\rho_f n d_{imp}^2}{\eta} \quad (1)$$

The power input P into an STR caused by the installed impellers turning in a fluid with the density ρ_f is estimated with

$$P = Po \cdot \rho_f \cdot n^3 \cdot d_{imp}^5 \quad (2)$$

which is used to determine the specific power input PV^{-1} containing the STR working volume V . In this study, the power number is estimated to be $Po = 6.5$ for both RT-6 and PBT-6 when used in combination. For biotechnological processes, including the cultivation of Chinese Hamster Ovary (CHO) cells, it is of interest to appraise critical length scales in such an STR to estimate potential cell damage. The most important is the Kolmogorov length scale λ_K , which describes the smallest scale in a turbulent flow and is calculated according to

$$\lambda_K = \left(\frac{\nu^3}{\bar{\epsilon}_T} \right)^{\frac{1}{4}}, \quad (3)$$

with the kinematic viscosity ν and the mean energy dissipation rate $\bar{\epsilon}_T$, which is defined for STRs like

$$\bar{\epsilon}_T = \frac{P}{V \rho_f} \quad (4)$$

Moreover, Ståhl Wernersson and Trägårdh [44] suggested in a scale-independent approach that the highest local energy dissipation rates $\epsilon_{T,loc}$ occur in the vicinity of the Rushton turbine and thus the length scales must be smaller according to

$$\log(\phi) = \log \left(\frac{\epsilon_{T,loc}}{\bar{\epsilon}_T} \right) = -1.04 \cdot \left(\frac{2 \cdot r_{imp}}{d_{imp}} \right) + 3.10, \quad (5)$$

where ϕ is the ratio between $\epsilon_{T,loc}$ and $\bar{\epsilon}_T$. In that case, the maximum value would occur with the radius $r_{imp} = 0.5 \cdot d_{imp}$ directly at the edge of the impeller, which is equal to $\phi = 114.8$ and would result in lower Kolmogorov length scales $\lambda_{K,\phi=115}$ (Table 2). Most recently, Šrom et al. report of $\phi = 4114$ in the same reactor setup, measured experimentally [13]. Literature findings suggest that cell damage is unlikely to occur if the Kolmogorov length scale exceeds the size of the respective cultivated cell, such as a CHO cell between 12 and 14 μ m in diameter [14,45,46].

Another length scale used in this study is the Taylor scale

$$L_E = u'_{rms,sim} \cdot \sqrt{\frac{15 \nu}{\bar{\epsilon}_T}}, \quad (6)$$

which helps to roughly estimate the size of the smallest eddies [47] with $u'_{rms,sim}$ being the root mean square of the fluid velocity fluctuations [48], which is taken from the numerical simulations and being roughly a tenth of the tip speed $u'_{rms,sim} = 0.1 \cdot u_{tip}$ [32] (Table 2).

2.2. Lagrangian sensor particles

The spherical Lagrangian Sensor Particles (LSPs) are engineered by the Helmholtz-Zentrum Dresden-Rossendorf in Germany (HZDR) (Fig. 2) [29]. They are composed of two half shells (Polyamid P4000, chemically polished), which are connected with a threaded rod and sealed

Table 2

Overview of volumetric power inputs PV^{-1} , stirrer Reynolds numbers Re_{STR} , tip speeds u_{tip} , root mean square of the fluid velocity fluctuations from simulations $u'_{rms,sim}$, estimated Taylor scales L_E based on the latter, Kolmogorov length scales λ_K and estimated minimum Kolmogorov length scales in the vicinity of the impeller tip $\lambda_{K,\phi=115}$.

Mixing parameter	Impeller frequency n / rpm			
	32	40	55	63
PV^{-1} / W m $^{-3}$	10.2	20.0	52.0	78.1
Re_{STR} ($\cdot 10^5$) / -	2.5	3.1	4.3	4.9
u_{tip} / m s $^{-1}$	1.1	1.4	1.9	2.2
$u'_{rms,sim}$ ($\cdot 10^{-1}$) / m s $^{-1}$	1.2	1.5	1.9	2.3
$L_E(u'_{rms,sim})$ ($\cdot 10^{-3}$) / m	4.6	3.9	3.2	3.1
λ_K ($\cdot 10^{-6}$) / m	95.7	81.0	63.8	57.6
$\lambda_{K,\phi=115}$ ($\cdot 10^{-6}$) / m	29.3	24.7	19.5	17.6

with an o-ring. With a diameter of $d_{LSP} = 40.0 \cdot 10^{-3}$ m their weight is approximately $m_{LSP} = 33.0 \cdot 10^{-3}$ kg including all internal electronics (microcontroller, pressure sensor, battery, etc.). Similarly to previous experiments [32], the current investigation exclusively evaluates pressure sensor data collected at a frequency of approximately 50 Hz, which are stored on an SD card onboard during the experiments and retrieved subsequently. The 24 bit stainless steel piezoresistive pressure sensor 89BSD (TE Connectivity) offers a measurement range of 0 to 6 bar with a pressure resolution $p_{res} = 9$ Pa (corresponds to approximately 0.9 mm in height) at an oversampling ratio of 4096 samples [49]. For each impeller frequency, at least 14 single LSP trials are analyzed. The corresponding particle volume fraction is $\phi_{LSP,exp} \leq 0.8 \cdot 10^{-5}$. The recording time of a single LSP in one trial is $t_{rec,single,exp} \leq 1$ h, totaling a measurement duration of 13.0 h for each dataset (see [Gitlab project](#)). The experimental data for each LSP run is preprocessed with a low-pass moving average filter of 15 consecutive values and, for comparability, interpolated with the *spline* method to a uniform 50.0 Hz signal, since the original sampling rates vary between 49.8 Hz and 50.0 Hz.

The LSP density calibration method, recently published in Hofmann et al. [32], is conducted identically in this study. Two glass vessels are prepared: the first contains the medium of interest from inside the STR, accessible through the bottom valve V-1/4 (Fig. 1), and the second is a sodium chloride (NaCl) solution adjusted to the upper allowed density limit (Table 1). By turning the upper half-shell of the LSP to modify the volume, and thus the density, it is adjusted to slowly sink in the first vessel and slowly rise in the second. The temperature of both vessels is kept constant with a water bath at the reactor temperature T .

2.3. Stokes number estimations and calculations

In process engineering and specifically in fluid mechanics, dimensionless numbers facilitate the comprehension, characterization, and prediction of an investigated system across varying scales. In numerous publications, the Stokes number St is defined as the ratio of the particle relaxation time to the characteristic flow time scale [50]

$$St = \frac{\tau_p}{\tau_f} \quad (7)$$

and is used to quantify the flow-following capability of particles within a fluid [51]. In the context of single-point flow statistics in turbulent particle-laden flows, Ouellette et al. suggest that particles are effective flow tracers, if the Stokes number is in the order of magnitude of $St \approx \mathcal{O}(10^{-2})$ [52]. Tropea et al. mention a sufficient level of accuracy in flow tracing when $St < 0.1$, with errors remaining below 1% [53]. In previous studies, it has been shown that the Stokes number applies as a reference point for the classification of LSPs in STRs, even for LSP sizes as used in this study, which is $d_{LSP} \lambda_K^{-1} \approx \mathcal{O}(10^3)$ [27,32].

Generally, the particle relaxation time

$$\tau_p = \frac{4}{3} \frac{\rho_p d_p^2}{\eta c_D Re_p} \quad (8)$$

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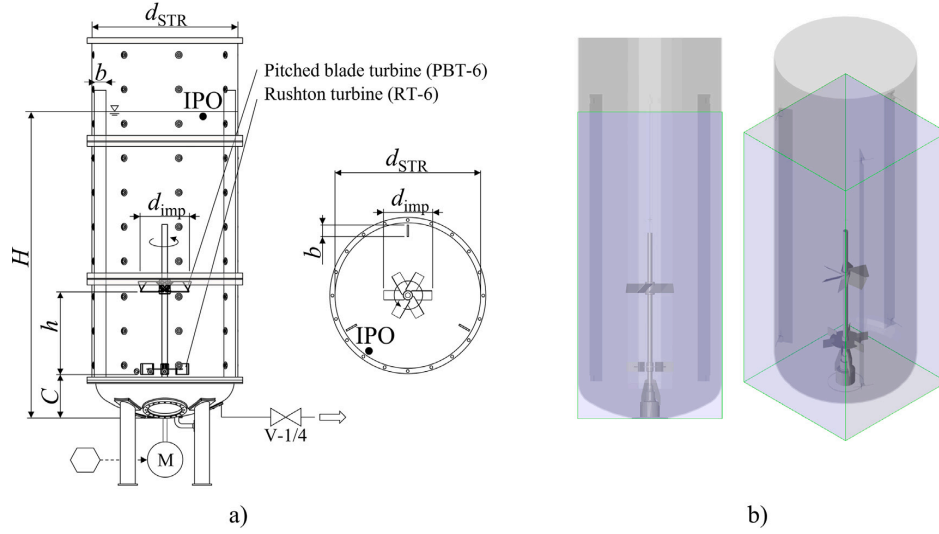


Fig. 1. (a) Schematic depiction of the acrylic glass reactor with a filling level of H , and a diameter of d_{STR} , equipped with three baffles of a width b . A Rushton turbine (RT-6, first stage) and a pitched blade turbine (PBT-6, second stage) with a diameter of d_{imp} are installed with a spacing of h and an off-bottom clearance of C . Fluid samples are taken from valve V-1/4. The location of the injection point (IPO) for hydrochloric acid and sodium chloride for the mixing time experiments is indicated with a black dot in the side view and the top view. (b) Schematic views of the STR with a green bounding box indicating the main lattice domain for the numerical simulations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



Fig. 2. Depiction of the LSP design by Buntkiel et al. [29] with the two connected half-shells (left), the top half-shell with its threaded rod (center), and the bottom half-shell including the electronics (right) [33].

with the particle density ρ_p , the particle diameter d_p , the dynamic viscosity η , the drag coefficient c_D according to Haider and Levenspiel [54], and the particle Reynolds number Re_p originates from the simplified version of the equation of motion in stationary flows [55]. This approach considers non-Stokesian drag, which plays a dominant role in the herein investigated Re_p due to the wake generated behind the particle [56]. In case of inertial particles, the particle Reynolds number

$$Re_p = \frac{\rho_f d_p |u - v|}{\eta}, \quad (9)$$

needs to be solved and contains the fluid density ρ_f and the slip velocity $|u - v|$, which are the relative velocities between the undisturbed fluid flow u and the LSP v .

The characteristic large eddy turnover time of the surrounding fluid

$$\tau_f = \frac{L}{u}, \quad (10)$$

with L being the characteristic fluid flow length scale of the undisturbed flow and u being the undisturbed carrier fluid velocity is challenging to determine experimentally on an industrial scale.

However, in terms of L , three approaches are presented in previous studies: it can be chosen as (i) the Taylor scale L_E (Eq. (11)) [32], (ii) the macro-scale of the turbulence, which is approximately the size

of the impeller diameter d_{imp} (Eq. (12)) [57], or (iii) a compromise between the turbulence length scale Λ (or the integral length scale) according to Ochieng et al. with $\Lambda = 0.1 d_{imp} = 0.067$ m [58] and the particle size ($d_p = 0.040$ m) on the one hand, and the Taylor scale $L_E(u'_{rms, sim})$ and the distance between the lattice points in the numerical simulation ($\Delta x = 0.004$ m) [34] on the other hand. A characteristic flow length scale of the mean flow $L_{MF} = 20 \cdot 10^{-3}$ m is chosen to represent a good compromise between the mentioned scales and being just within the inertial subrange on the one hand and a high resolution on the other (Eq. (17)).

With regard to velocity, especially slip velocity, two approaches can be chosen: (i) using the simplified method, which takes the difference of the tip velocity u_{tip} and the maximum average axial velocity from the experiments into account as $u_{tip} - |\bar{v}|_{z, max}$, or (ii) using the velocity vectors from the LB LES.

In terms of the first approach, considering experimental results, this concludes to three simplified temporal scales

$$\tau_{f, micro-scale} = \frac{L_E}{u_{tip}} \quad [32], \quad (11)$$

$$\tau_{f, meso-scale} = \frac{d_{imp}}{u_{tip}} \quad [28], \quad (12)$$

and

$$\tau_{f, macro-scale} = \bar{t}_{circ, overall} \quad [28], \quad (13)$$

where the latter accounts for the macroscopic effects and utilizes the overall average circulation time $\bar{t}_{circ, overall}$ of LSPs in an STR, similarly to the understanding of the global mixing time [59,60]. With the knowledge that the root mean square of the fluid velocity fluctuations is approximately $0.1 \cdot u_{tip}$ in this STR, Eq. (6) and (11) are further derived to

$$\tau_{f, micro-scale} = 0.1 \cdot \sqrt{\frac{15 \nu}{\epsilon_T}}. \quad (14)$$

The second approach takes only numerical simulation results into account in order to solve Eqs. (9) and (10). To do so, Bellani et al.

present one approach with a stochastic slip velocity of large neutrally buoyant particles [61]. They suggest

$$V_{\text{slip, stoch}} = \sqrt{u'^2 - \bar{v'^2}}, \quad (15)$$

where the primes account for the velocity fluctuation relative to the average value, e.g. for the radial fluid velocity component $u'_{r,i} = u_{r,i} - \bar{u}_r$. Hence, the stochastic fluid velocity fluctuation magnitude of the tracer is defined as

$$u_{\text{stoch}} = \sqrt{u'^2}, \quad (16)$$

which is also presented in this study for comparison.

In order to obtain spatially resolved Stokes numbers for the mean flow (MF) [48] throughout the reactor, the numerical simulations presented herein provide 3-dimensional information about the velocities $\mathbf{u}$ of the fluid and $\mathbf{v}$ of the LSPs. Therefore, the euclidean norm $\|\cdot\|$ of the slip velocity vector and the fluid velocity vector is considered, along with the characteristic length scale of the mean flow L_{MF} , which results in

$$St_{\text{MF}} = \frac{4}{3} \frac{\rho_p}{\rho_f} \frac{d_p}{\|\mathbf{u} - \mathbf{v}\|_2} \frac{\|\mathbf{u}\|_2}{L_{\text{MF}}}, \quad (17)$$

and is similarly used in a previous study on laboratory scale with inertial, finite-sized particles in a 4D-Particle Tracking Velocimetry (4D-PTV) setup with accompanying numerical simulations [34].

2.4. Lattice-Boltzmann simulations

The numerical simulations are conducted with M-Star CFD (M-Star Simulations, LLC, Version 3.10.32), which utilizes a Boltzmann solver to essentially numerically solve the Navier Stokes equation through the use of the Boltzmann transport equation as

$$\frac{\partial f}{\partial t} + \zeta^T \nabla_x f + \mathbf{F}_{\text{ext}}^T \nabla_\zeta f = \Omega(f) \quad [8,38,62,63]. \quad (18)$$

The equation is spatiotemporally discretized for a D3Q19 set of lattice points (Fig. 3). The Boltzmann equation consists of f , which is the time derivative of the particle distribution, ζ as the velocity and $\mathbf{F}_{\text{ext}}$ as the external forces of the D3Q19 model. The right side of the equation includes $\Omega(f)$, the collision parameter, for which the Bhatnagar–Gross–Krook parameter

$$\Omega(f) = -\frac{1}{\tau_c} \cdot (f - f^{\text{eq}}) \quad (19)$$

is used [62,64–66]. It is in the collision parameter where the collision time τ_c and f^{eq} are included. The variable quantifies the time between particle collisions and the equilibrium particle distribution, respectively. This allows for the solution of the Boltzmann equation

$$f_i(\mathbf{x} + \mathbf{c}_i \Delta t, t + \Delta t) = f_i(\mathbf{x}, t) + \Delta t \Omega_i(\mathbf{x}, t) \quad [8,62,64–66]. \quad (20)$$

The bounding box with green edges in Fig. 1 (b) highlights the main lattice domain of the STR, while the blue shade within the box denotes the respective filling height H to achieve the $V = 12.5 \text{ m}^3$ filling volume. To guarantee the validity of the study, an extensive grid independence study is conducted. The results are given in Appendix A. A mesh with 500 lattice points (LX 500) across the horizontal diameter and 1062 lattice points along the vertical axis of the STR is found to be the ideal trade-off between accurately calculating small eddies, not receiving physically unlikely results, and calculation capacity of the respective cluster hardware. The total number of lattice sites is equivalent to around 265.5 million across the main lattice domain. The lattice spacing, particularly the spacing across the impeller diameter, is consistent with the recommended number of lattice sites for a Rushton turbine and a PBT-6 configuration, guaranteeing grid independence [42]. Furthermore, the preliminary grid independency study coincides with the results proclaimed by Šrom et al. who worked with the same STR but applied refinement zones for areas of higher

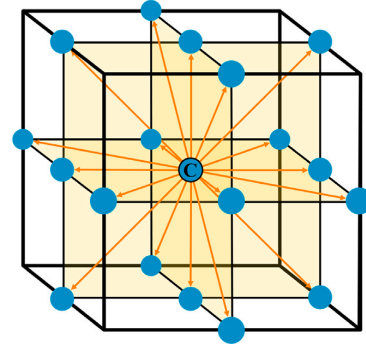


Fig. 3. Discrete velocity vectors pointing from the center lattice point (C) to all nearest neighbors in a D3Q19 Lattice-Boltzmann model structure.

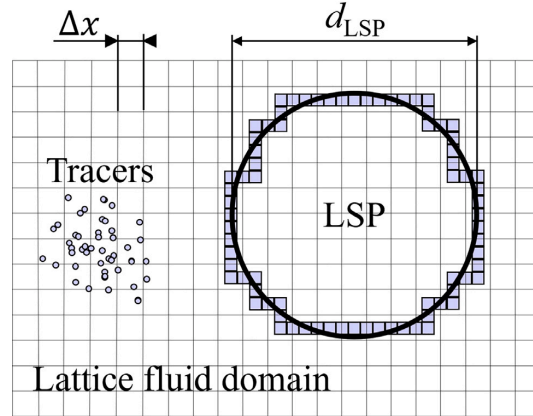


Fig. 4. Exemplary 2-dimensional depiction of the lattice domain with a lattice spacing of Δx , showing the two types of simulated particles used in this study: non-inertial, non-interacting point objects referred to as flow tracers (*tracer, sim*, left), and an LSP (*LSP, sim*, right) treated as a discrete element particle (thick circle demonstrates a set of Lagrangian points). For the latter and as a reference, the coarse computational boundary (squares) based on bounce-back rules of the LB method is shown according to Feng and Michaelides, but not used in this study [41].

turbulence [13]. The application of such refinements is not applied in the present study, as for the use of resolved particles nested in the application of the immersed boundary method, a constant set of contact points with the lattice structure provided more stable results. Additionally, the resolution in this study surpasses the recommended refinement required for a minimum resolution of 80% of the kinetic energy in the bulk of the STR [13,67].

For the study, a Courant number of $Co = 0.02$ was set with the fluid surface set to single phase under free slip conditions. The remaining system boundaries are set to no slip. The large eddy simulation model (LES) is applied as a turbulence model with a Smagorinsky coefficient of $C_s = 0.1$.

The simulated time for each operating point is a total of 515 s, with the first 35 s being disregarded because the STR is still reaching steady state during this period of time. Statistics and output files are saved with a frequency of 50 Hz once steady state is reached, which is a total simulated time of $t_{\text{sim, max}} = 480 \text{ s}$.

The particles simulated in the study are simulated as spheres (*LSP, sim*), set to the same diameter ($d_p = 0.04 \text{ m}$), replicating the LSPs used by Buntkiel et al. and Hofmann et al. [29,32]. The density of the

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particles is set at $\rho_p = 999.8 \text{ kg m}^{-3}$, to match the work of Buntkiel et al. The idea behind the resolved particles is that with sufficient coverage of the particle surface through various lattice points, the physical characteristics of the particle can be deduced from the solver itself, through the immersed boundary Lattice-Boltzmann method [41]. In the current case, the lattice spacing of 0.004 m guarantees that each LSP has approximately ten lattice sites over its diameter d_{LSP} (Fig. 4). From steady state on, output statistics for resolved particles are saved every 0.02 s. The initial release of the particles takes one second into the simulation, allowing for an even distribution starting point for the output statistics after steady-state is attained. A total of 98 LSPs mimicking resolved particles are simulated, amounting to a total of 13.0 h in data obtained from a Lagrangian perspective per rpm case, matching the amount of recorded lifeline data from the experimental work. The solid-particle interaction is set to bounce, allowing the particles to come in contact with the impellers; the particle-particle interaction is set to Hertz simple, following the standard settings of M-Star for a nonelastic collision of particles [42].

The preliminary simulations pertaining to the grid independence were conducted on a local cluster consisting of two AMD Epyc 7702 64 core processors and two NVIDIA Tesla A100 with 40 GB memory each. The further resolved particles and lifeline simulations were conducted on the high-performance computing cluster of the Hamburg University of Technology, using two AMD Epyc 9124 and two NVIDIA Tesla H100 GPUs, with wall clock times ranging from 5 to 7 days depending on the simulated operating point.

Before being used in the evaluation algorithm, the simulated data are checked in three steps to ensure meaningfulness and comparability with the experimental data in the identical geometrical vessel. First, any dataset in which 50 data points in total (corresponding to 1 s physical time) have values higher than the experimental filling level or lower than the lowest point in the reactor is discarded. Second, any dataset in which 250 consecutive data points (corresponding to 5 s physical time) have an axial distance of 0.04 m to the filling level are treated as *floating*, and thus are discarded. Third, any dataset whose correlation matrix has a pairwise linear correlation coefficient $r > 0.98$ and a respective p -value < 0.02 is also discarded, as this indicates identical datasets. The remaining datasets of each rpm case are reduced to a duration of $t_{\text{rec,tot,sim}} = 13.0 \text{ h}$.

The simulations of the resolved particles (*LSP,sim*) that mimic the experimental LSPs (*LSP,exp*) are further accompanied by the addition of non-inertial, non-interacting point objects referred to as flow tracers (*tracer,sim*) providing a full Lagrangian representation of the undisturbed fluid field. The steps and criteria for using the data follow those for the resolved particles.

2.5. Utilization of the pressure sensor data and corresponding simulation data

In this study, data from three different particle types is evaluated: experimental data of LSPs (*LSP,exp*) is compared to the data of simulated LSPs (*LSP,sim*) and simulated undisturbed fluid flow tracers (*tracer,sim*) in the same reactor setup. To do so, the time-dependent axial position

$$z(t) = H - \frac{p(t) - p_0}{\rho_f g} \quad (21)$$

of an experimental LSP is determined by using the recorded hydrostatic pressure p , the ambient pressure p_0 before each trial for each LSP, the fluid density ρ_f (Table 1), and the gravitational acceleration g [29,32].

Deriving these Lagrangian data points by time, the Lagrangian axial velocity

$$v_z(t) = \frac{dz(t)}{dt} \quad (22)$$

is obtained. Furthermore, by subdividing the reactor into 42 vertical slices j , each with a height of $h_j = 0.1 \text{ m}$, Eulerian data such as the probability of presence for each bin

$$P_j = \frac{N_j}{\sum_{j=1}^{42} N} \cdot 100\%, \quad (23)$$

where N_j are the counts in the respective bin j , as well as axial velocity distributions for $v_{z,\text{neg}}$ (negative, downward movement) and $v_{z,\text{pos}}$ (positive, upward movement) over the reactor height are derived. However, single-point Lagrangian axial velocity statistics are also presented in this study based on absolute velocities $|v|_j = v_{z,\text{mag}}$ and are depicted as probability density functions (PDF). The 3-dimensional location and velocity data from the numerical simulations are taken and evaluated directly with the same sampling rate as in the experiments.

For the hydrodynamic characterization of the STR, the circulation time distribution t_{circ} (CTD) over the reactor height and the hydraulic mean residence time distribution τ (RTD) over the reactor height are calculated for all three particle types. Both evaluations allow for the qualitative analysis of axial hydrodynamic compartments. Therefore, for the CTD, the time differences $t_{\text{circ},j,\Delta t}$ are calculated based on the duration it takes a particle to cross the central plane of slice j from top to bottom or from bottom to top, first at the initial time $t_{\text{circ},j,t=0}$ and subsequently at $t_{\text{circ},j,t>0}$ [28,32]. Regarding the RTD, the particle volume flow rates $\dot{V}_j$ in the respective reactor volumes V_j with their surface areas A_j are calculated according to

$$|\vec{V}|_j = |\vec{v}|_j \cdot A_j, \quad (24)$$

eventually leading to the particle hydraulic residence time

$$\bar{\tau}_j = \frac{\dot{V}_j}{|\vec{V}|_j}. \quad (25)$$

Due to partly different recording lengths of the trials or counts over the reactor height, a weighted average of the value x is determined as

$$\bar{x} = \frac{\sum_{i=1}^M w_i x_i}{\sum_{i=1}^M w_i}, \quad (26)$$

with the weight w of the respective trial i of total M trials per dataset. Corresponding uncertainties of the mean $\Delta_{\bar{x}}$ are based on the calculation of the standard deviation of the individual measurement from the mean Δ_x and, if applicable, an error propagation. For a detailed documentation of the calculations the reader is referred to the [Gitlab repository](#).

An investigation of the dynamic pressure acting on the LSP and the resulting height error is discussed in [68] with similar designs. The experimental results presented herein in combination with the numerical simulations come to a similar conclusion that those are negligible. Even at the highest impeller frequency of $n = 63 \text{ rpm}$ ($u_{\text{tip}} = 2.2 \text{ m s}^{-1}$), the estimate of the maximum dynamic pressure fluctuation at the tip of the impeller is approximately 128 Pa, which corresponds to a relatively low height tolerance of approximately 13 mm compared to the bin size $h_j = 100 \text{ mm}$.

2.6. Particle volume fraction sensitivity study for numerical simulations

Due to the complexity of the resolved particle LB LES utilized herein and the associated demanding wall clock time, it is essential to obtain the maximum possible amount of data within the approximately 210 h available on the high-performance cluster. However, a numerical replica of the experimental trials with a perpetual physical time of 1.0 h per LSP with a maximum of three LSPs in the STR is not possible. This is the reason why 98 particles are eventually used, each with $t_{\text{sim,max}} = 480 \text{ s}$ physical time, totaling 13.0 h of data for each particle type, *LSP,sim* and *tracer,sim*. Those 98 LSPs are the result of the aforementioned elimination algorithm (Chap. 2.4), but initially there are approximately $N_{\text{LSP,sim}} = 250$ LSPs in the STR.

Multiple studies have extensively focused on characterizing turbulence attenuation in particle-laden flows [61,69–71]. Due to the large number of inertial, finite-sized, neutrally buoyant particles in the LB LES, a sensitivity study is carried out at 55 rpm, scrutinizing the decrease in turbulence of the carrier fluid, as well as particle perturbation and collision, as a function of five LSP quantities, $N_{\text{LSP,sim}} \in \{18, 44, 92, 155, 304\}$, corresponding to volume fractions $\Phi_{\text{LSP,sim}} \in \{0.5, 1.2, 2.5, 4.2, 8.1\} \cdot 10^{-4}$. For each case, and additionally for a run without LSPs, 660 tracers are submerged in the same run together with the different number of LSPs to record a potential turbulence attenuation of the fluid. Each particle type is recorded for a physical time of 60 s at a frequency of 50 Hz, totaling 11.0 h tracer data.

One approach is described by Crowe et al. [50], which helps to classify the dispersed phase flow in the STR as *dense* or *dilute*. If the ratio of the particle response time τ_p to the time between collisions τ_c is

$$\frac{\tau_p}{\tau_c} < 1, \quad (27)$$

the flow is considered as dilute, since the particles have sufficient time to adjust to local fluid dynamic forces. The time between the collisions is defined as

$$\tau_c = \frac{1}{\frac{N_{\text{LSP,sim}}}{V} \pi d_p^2 V_{\text{slip,stoch}}}. \quad (28)$$

The second approach examines the velocity fluctuations u'_{rms} of all three velocity vector components (in cylindrical coordinates) as a function of the volume fractions according to Cisse et al. and Bellani et al. [72,73] in the form of

$$u'^2_{\text{rms}} = \overline{(u_i - \bar{u}_i)^2}, \quad (29)$$

with u_i being a single fluid velocity vector in time, and $\bar{u}_i$ representing the average over time. Moreover, the turbulent kinetic energy (TKE)

$$k = \frac{1}{2} (u'^2_{z,\text{rms}} + u'^2_{r,\text{rms}} + u'^2_{\theta,\text{rms}}) \quad (30)$$

is used to potentially determine a decrease with an increasing particle number.

The third approach is presented by Ouellette et al. [70] and Hofmann et al. [34] as single point statistics via a probability density of all Lagrangian velocities occurring in comparison to the undisturbed fluid flow tracers.

2.7. Global mixing times and local mixing time distributions

Regarding the detailed description of the determination of the global mixing time, the reader is referred to the previous study [32]. The decoloration method with bromothymol blue (BTB) is conducted with the addition of a 2 M hydrochloric acid (HCl) and a 2 M sodium hydroxide (NaOH) solution during the LSP trials. The injection point of HCl (IPO) is located 13° behind the baffle in the impeller's rotational direction (Fig. 1). The injection lasts 23 s with the 250 mL HCl solution mixed with an aqueous 3 L sodium chloride (NaCl) solution of 312.5 g L⁻¹ in deionized water (DW). The latter has no influence whatsoever on the trials described herein. Uniform lighting is provided by eight LED panels and a white diffuser fabric positioned behind the reactor. The decoloration is recorded at a frequency of 30 Hz using a NIKON D7500 camera with a SIGMA lens (1:1.8, 20 mm f22.0) at a resolution of 1920 × 1080 pixels. Post-processing and the evaluation of the mixing times is performed by an in-house developed MATLAB algorithm, which utilizes the trend of normalized gray scale values over time. By definition, the global mixing time criterion of 95% is achieved once the normalized sensor output reaches and remains between 0.95 and 1.05 of its final value [74]. In this study, each mixing time experiment is conducted in triplicates for the impeller frequencies $n \in \{24, 32, 40, 55, 63\}$ rpm. Hence, the normalized sensor output over time is the gray scale intensity I'_t , which is plotted as the log variance

$$\log \sigma_t^2 = \log((I'_t - 1)^2) \quad (31)$$

over time.

However, in order to determine heterogeneities in the form of compartments, it is insufficient to evaluate the entire reactor as a single entity and obtain only the global mixing time $\Theta_{\text{glob},95}$. Therefore, as shown in a previous study [59], for a 2 × 2 pixel array throughout selected gray scale images over time, a respective mixing time is calculated, which yields the local mixing time $\Theta_{\text{loc},95}$. For this purpose, a *moving average* filter is used to calculate the smoothed gray values. Eventually, the relative local mixing time is plotted as a ratio of

$$\Theta^* = \frac{\Theta_{\text{loc},95}}{\Theta_{\text{glob},95}}. \quad (32)$$

3. Results and discussion

3.1. Numerical simulation sensitivity study

The sensitivity study focuses exclusively on the Lattice-Boltzmann large eddy simulations (LB LES) data with different numbers of Lagrangian Sensor Particles (LSPs) in the stirred tank reactor (STR), and examines the influence of large, nearly neutrally buoyant particles with a diameter of $d_{\text{LSP}} = 40$ mm on the intensity of turbulence fluctuations. This necessitates a dilute flow so that disturbances in the carrier fluid remain isolated without interference [72]. According to Crowe et al. [50], all particle volume fractions examined in this study meet the criterion that the ratio of the particle response time to particle collision time $\tau_p \tau_c^{-1} < 1$, being far below 0.1 (Table 7 in Appendix B). These estimations use the stochastic slip velocity $V_{\text{slip,stoch}} \approx 0.135$ m s⁻¹ and the calculated values for $\tau_p \approx 1.0$ s, such as the drag coefficient $c_D \approx 0.4$ and the particle Reynolds number $Re_p \approx 5.7 \cdot 10^3$. Nevertheless, direct particle interactions are observed through collisions or perturbations within a distance of their respective boundary layer of d_p [75] by calculating the probability of particle collision or perturbation (PCP). The results demonstrate a PCP between 3.75% and 7.33% for the particle number used in the main study (Fig. 5).

Highlighting the fluid velocity fluctuation variance u'^2_{rms} , which shows the instantaneous fluctuation from the mean, a decrease can be observed for the three velocity components with an increasing particle volume fraction $\Phi_{\text{LSP,sim}}$ (Fig. 5). Literature findings confirm this behavior, as well as a proposed fitting to $(1 - (3.75 \cdot \Phi_{\text{LSP,sim}})^{2/3})$ (gray dashed trends), despite the use of higher particle number densities [72]. Moreover, a decrease of the turbulent kinetic energy (TKE) k of approx. 10% between the lowest and highest volume fraction is shown, which also pertains to the findings of Bellani et al. albeit for a lower particle Reynolds numbers $Re_p = 175$ and $\Phi_{\text{LSP,sim}} = 1.4 \cdot 10^{-3}$ relative to single phase [73]. The single-point velocity statistics of the fluid appear not to be affected by the presence of LSPs (Fig. 18 in Appendix B).

These findings indicate that at the particle volume fraction used in the main study, the LSPs already substantially perceive the presence of neighboring particles through alterations in the flow and through collisions that occur. On a small scale, no significant turbulence disturbance can be observed, whereas a slight attenuation is seen globally. This entails in a general decrease in the velocity of the simulated LSPs because of the substantial volume fraction. This impact is specific to the simulations, as the experiments utilize significantly lower volume fractions.

3.2. Axial velocity distributions

The first visualization presents information about the velocity of all three particle types (experimental LSPs, simulated LSPs and tracers). To do so, the derivative of the height position over time $dz(t) dt^{-1}$ yields the axial velocity $v_z(t)$ along the trajectory of the particle. Binning the datasets of the three particle types over the reactor height in 42 slices j provides information of the average axial velocity distribution for both

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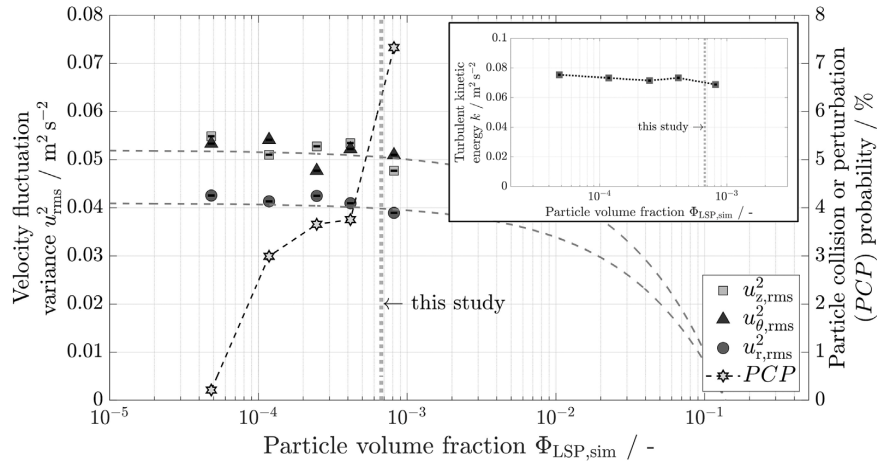


Fig. 5. Left y-axis: Velocity fluctuation variances for the three cylinder coordinate components $u_{z,rms}^2$ (squares), $u_{\theta,rms}^2$ (triangles), and $u_{r,rms}^2$ (circles) as a function of different particle volume fractions $\Phi_{LSP,sim}$ for $n = 55$ rpm. The dashed lines are a fit to $(1 - (3.75 \cdot \Phi_{LSP,sim})^{2/3})$. Right y-axis: Particle collision or perturbation PCP probability (hexagram) within a distance of a boundary layer of d_p . Inset: Turbulent kinetic energy (TKE) k as a function of $\Phi_{LSP,sim}$ for $n = 55$ rpm. Vertical dashed lines represent the volume fraction used in the main study.

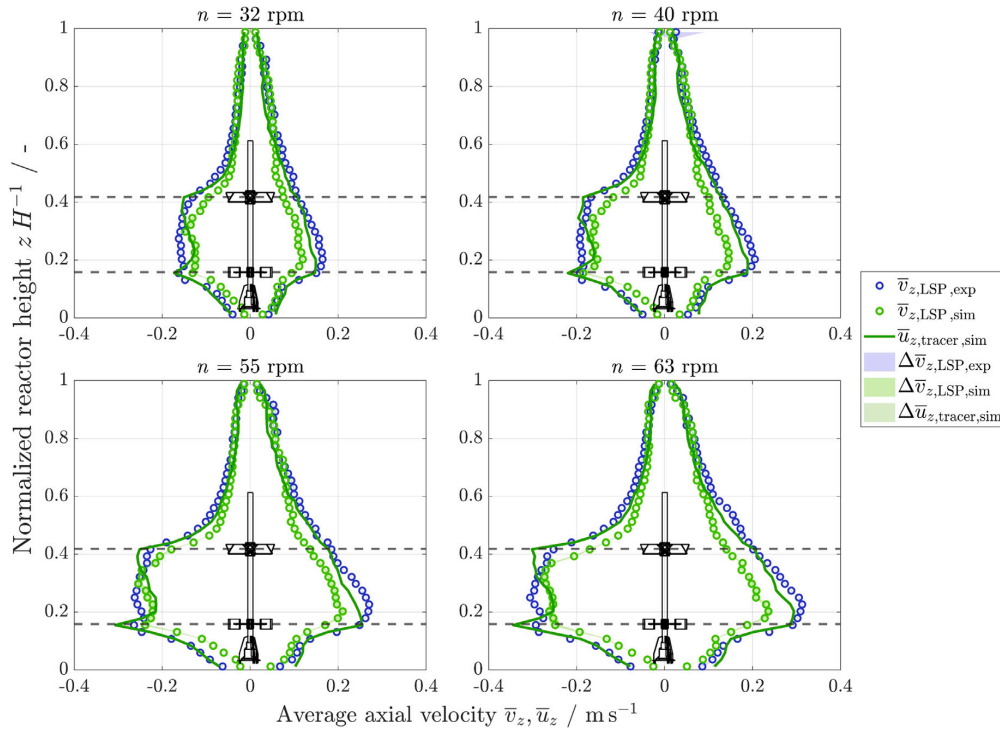


Fig. 6. Average positive (upwards flow) and negative (downwards flow) axial LSP velocity $\bar{v}_z$ and tracer velocity $\bar{u}_z$ distributions as a function of the normalized reactor height z ($H = 4.2$ m) for experimental LSPs (LSP,exp , blue circles), simulated LSPs (LSP,sim , green circles), and for simulated tracer ($tracer,sim$, green line), each for the rpm cases $n \in \{32, 40, 55, 63\}$ rpm. The vertical bin size is 10 cm. The shaded areas depict the uncertainty Δ of the respective dataset. The gray dashed lines indicate the center position of the RT-6 (lower) and PBT-6 (upper). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

negative (downward movement) and positive (upward movement) velocities $\bar{u}_z$ and $\bar{v}_z$ of the tracers and the LSPs, respectively. Fig. 6 reveals the characteristic profiles of the four rpm cases $n \in \{32, 40, 50, 63\}$ rpm.

Generally, all trends show the highest average axial velocities at the height of the impellers (gray dashed lines), and with increasing

distance from them, the velocity decreases. The undisturbed tracer particles (green line) have a sharp peak at both impeller positions in the downward direction, whereas upwards a more rounded peak becomes apparent at the RT-6. Above the latter ($z/H^{-1} \approx 0.25$), a distinguished suction zone toward RT-6 is recognizable by a sudden velocity decrease

in the downward direction. At first glance, the experimental LSPs (blue dots) seem to have the overall highest average axial velocity. However, between the RT-6 and the reactor bottom and in the aforementioned peak zones at both impellers, their average axial velocity is less than that of the tracers. The higher the impeller frequency, the more distinct the negative velocity drop in the suction zone above the RT-6. The simulated LSPs (green dots) show a trend similar to the experimental ones, especially in the upper third of the reactor. Nevertheless, the maximum average axial velocity difference between the simulated and experimental LSPs amounts to $\approx \{0.05, 0.06, 0.08, 0.10\} \text{ m s}^{-1}$ for all impeller frequencies, respectively, in terms of positive average axial velocities at the height of $z H^{-1} \approx 0.21$.

The prominent velocity difference between experimental and simulated LSPs is most likely due to the number of simulated LSPs in the reactor, whose effect on velocity is explained in the sensitivity study (Chap. 3.1). Another reason is found in the next Section 3.3, where the effects of a different center of mass on the LSP's velocity is elucidated. Although the utilized LSP,exp only have a mass offset of $r_{shift} = 1.15 \text{ mm}$ toward their geometrical center, they seem to break out easier of the upper less turbulent zone and thus show a faster vertical movement in combination with a higher probability in the zone between the impellers. The LSP,sim are more likely to be slowed down in the axial direction due to an easier facilitation of a horizontal velocity transition. The decrease in TKE of the fluid between 3% and 10% compared to the experimental runs, and additionally, the PCP probability between 3% and 7% add up to the difference shown in the axial velocity.

Another possibility of depicting axial velocities is presented in Fig. 7 for $n = 55 \text{ rpm}$. This statistical evaluation in the form of a probability density function (PDF) uses all the Lagrangian velocities of the tracers $u_{z,mag}$, and the LSPs $v_{z,mag}$, as absolute values, and can be used for an unbiased plausibility confirmation of the obtained data. The upper depiction shows the obtained velocities in the entire reactor, whereas the center and lower depiction show the velocities in the high turbulent zones, at the height of the RT-6 and the PBT-6, respectively. The probabilities for the last two are normalized due to a different number of data points. The tip speed v_{tip} is marked with a vertical dashed line. Because the axial distribution of the tracers throughout the reactor is sufficiently homogeneous, it is used as a reference for this evaluation. Basically, the tracers show a higher probability at velocities of 0.9 m s^{-1} and above (upper and lower), or even of 0.75 m s^{-1} and above (center) than those of LSP,exp and LSP,sim , which is obvious, since they represent the undisturbed fluid. The highest velocities for both LSP types occur at the height of RT-6 (center) and PBT-6 (lower) with an overlapping trend starting at 0.8 m s^{-1} , demonstrating their strong, but similar inertial behavior compared to the tracers in high turbulent zones. In those zones, the trends for slow velocities are also in good agreement, most likely representing the upward movement at the reactor walls or near the baffles. However, for LSP,sim , there is a clear probability gap around $\approx 0.5 \text{ m s}^{-1}$, which is mainly due to their 3-fold lower probability of presence in the impeller zones compared to LSP,exp . Instead, LSP,sim show good agreement with the tracers at very low velocities up to 0.1 m s^{-1} , due to their similar probability of presence in the upper half of the STR at low velocities. In total, LSP,sim have an approximate 78% probability of staying in such a low turbulent zone, compared to 40% for LSP,exp , which confirms the conclusion of the work of Rautenbach et al. [33] once again.

3.3. Vertical probabilities of presence

Moreover, for all three particle types (experimental LSPs, simulated LSPs and tracers), the height distribution during the trials is investigated. This information is pivotal, as all results, especially direct comparisons, are based on the location and the time each particle spent there.

Fig. 8 depicts the probabilities of presence over the normalized reactor height for the four rpm cases $n \in \{32, 40, 55, 63\} \text{ rpm}$. The

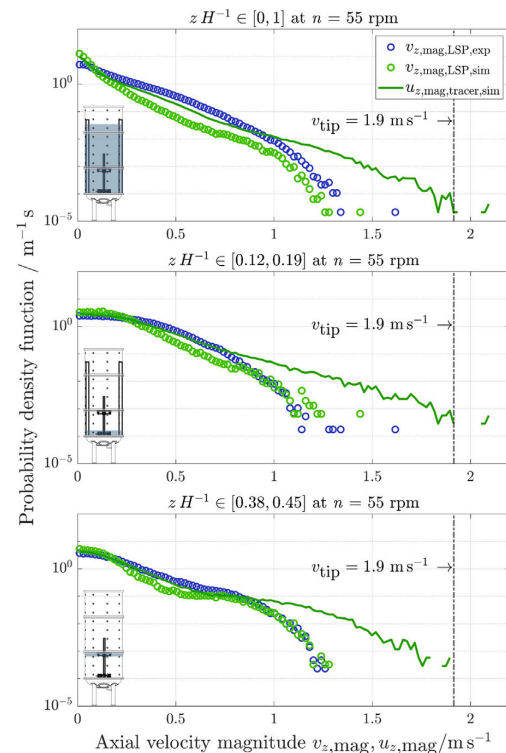


Fig. 7. Probability density function of Lagrangian axial LSP velocity magnitudes $v_{z,mag}$ (experimental: blue circles; simulated: green dots) and tracer velocity magnitudes $u_{z,mag}$ (green line) at $n = 55 \text{ rpm}$. Upper: all axial velocities; Center: axial velocities at the height of the RT-6 (respectively normalized); Lower: axial velocities at the height of the PBT-6 (respectively normalized). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

undisturbed tracer particles ($tracer,sim$, green line) are nearly homogeneously distributed over the height. Generally, with an increasing impeller frequency their distribution trend gets more even throughout the reactor height. The characteristic behavior at the bottom is due to the dish shape of the reactor. Although experimental LSPs (LSP,exp , blue dots) seem to accumulate in the bottom of the reactor at 32 rpm, at higher frequencies, they tend to distribute well up to the pitched blade turbine (PBT-6, top dashed line). Above that impeller, the distribution drops to zero toward the top of the STR for all rpm cases. The simulated LSPs (LSP,sim , green dots) demonstrate the opposite behavior. Their distribution between the impellers is much lower than those of the experimental LSPs, almost mirrored at the tracer distribution trend. They show an even stronger accumulation at the very bottom of the reactor below the normalized height of $z H^{-1} \approx 0.1$ for all impeller frequencies, but a similar distribution as the tracers above the PBT-6. At the very top of the reactor they as well reveal a clustering behavior.

Even though the overall $2.34 \cdot 10^6$ gained data points for each rpm case are almost tripled compared to the previous study [32], the impeller setup used herein reveals a large, less turbulent zone in the upper half of the STR, which is clearly seen by the uneven trend of the tracers. This characteristic would necessitate an even larger dataset, in particular if mass transfer or heat transfer phenomena need to be examined in detail in the entirety of the STR for low impeller frequencies. For our study, the tracer data is sufficient with a low uncertainty and serves as a point of reference when analyzing LSP data. For 40 rpm and above, the LSP,exp trend at the Rushton turbine

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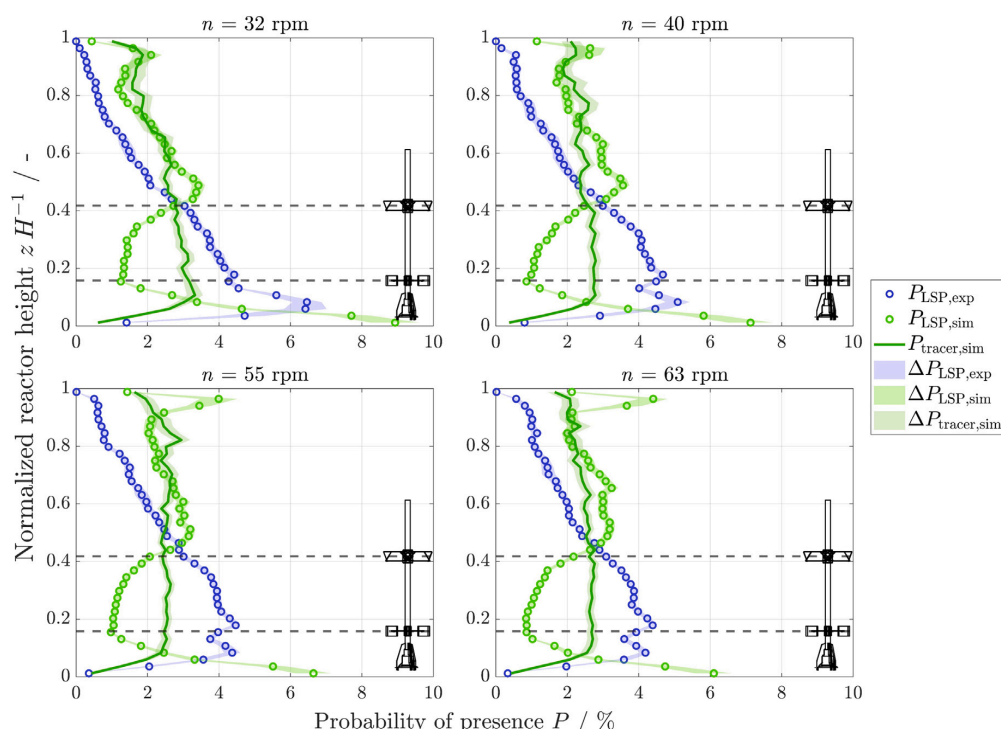


Fig. 8. Axial probability of presence P as a function of the normalized reactor height $z H^{-1}$ ($H = 4.2$ m) for experimental LSPs (LSP,exp , blue circles), simulated LSPs (LSP,sim , green circles), and for simulated tracer ($tracer,sim$, green line), each for the rpm cases $n \in \{32, 40, 55, 63\}$ rpm. The vertical bin size is 10 cm. The shaded areas depict the uncertainty Δ of the respective dataset. The gray dashed lines indicate the center position of the RT-6 (lower) and PBT-6 (upper). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(RT-6, lower dashed line) is similar to a study published by Bisgaard et al. [76], with a sudden increase just above and below the impeller, which is explained by the formation of trailing vortices behind the impeller blades [77]. The general decline in the probability of presence toward the top is explained by the less turbulent zone in the upper half of the STR and the small but perceptible buoyancy difference of $(\rho_f + 1.0) \text{ kg m}^{-3}$. The density difference of the simulated LSPs is set slightly higher at $(\rho_f + 1.7) \text{ kg m}^{-3}$, since initial numerical attempts failed due to a large number of floating LSPs. This behavior was not observed in experiments. Interestingly, in terms of density differences, Lauterbach et al. found similar results in their CFD analysis. Using a Poincaré map, they demonstrated the best homogeneous distribution with an even higher particle density $(\rho_f + 50) \text{ kg m}^{-3} \leq \rho_p \leq (\rho_f + 100) \text{ kg m}^{-3}$ in a 2 L bioreactor [78]. However, their setup barely resembles the parameters used in this study. Hence, the balanced density difference used herein is chosen to minimize floating particles and reduce bottom accumulation in the reactor. As reported in the literature for particle-laden flows, most likely the particles already tend to cluster and collide in those regions with a prevailing characteristic Stokes number [79,80].

The characteristic behavior of the probability of presence of the LSP,sim can be explained by the results of a study by Rautenbach et al. [33]. Therein, the particle dynamics of two types of Lagrangian Particles (LP) of the same size ($d_p = 40$ mm) and density are experimentally investigated in a flow passage at similar fluid flow speeds. In one LP, the center of mass is offset by $r_{shift} = 6.50$ mm. In the second LP, the center of mass is located at the equatorial geometric center. Basically, those two LP types correspond to the two LSP types used in this study (experimental and numerical), since for LSP,exp the center of mass is offset by $r_{shift} = 1.15$ mm due to the arrangement of the internal hardware components [29], and LSP,sim are spheres with their center

of mass at their geometric center. The three principal conclusions of their work are directly transferable to this study:

1. Particles with a centrally situated center of mass, like the LSP,sim , show a more homogeneous probability of presence with a higher presence in the less turbulent region.
2. To perform horizontal flow direction changes, particles with an offset center of mass, such as LSP,exp , require stronger momentum. Hence, they are more likely to break out of the main flow in the direction of the gravitational force.
3. Particles with a centrally situated center of mass, like the LSP,sim , show an overall slower velocity and a higher likelihood of changing their flow direction, as they tend to convert kinetic energy into rotational energy, which slows them down. Especially the axial velocity is decreased.

These findings support the results described herein for the LSP,sim , such as the higher probability of presence in the less turbulent upper region of the STR, and their ability to remain suspended in this region due to their increased likelihood of being reflected by the turbulent zone occurring in the discharge flow of PBT-6 and RT-6. From a statistical point of view, it is worth mentioning that both datasets LSP,exp and LSP,sim represent a non-ergodic system. Although they have the same number of data points, they differ in the number of LSPs, their respective trial time, and distance traveled in the reactor (Table 3), which could also be a factor in the presented results.

3.4. Circulation time distribution

The evaluation of the circulation time distribution (CTD) is a widely used method to obtain information about the axial homogeneity of

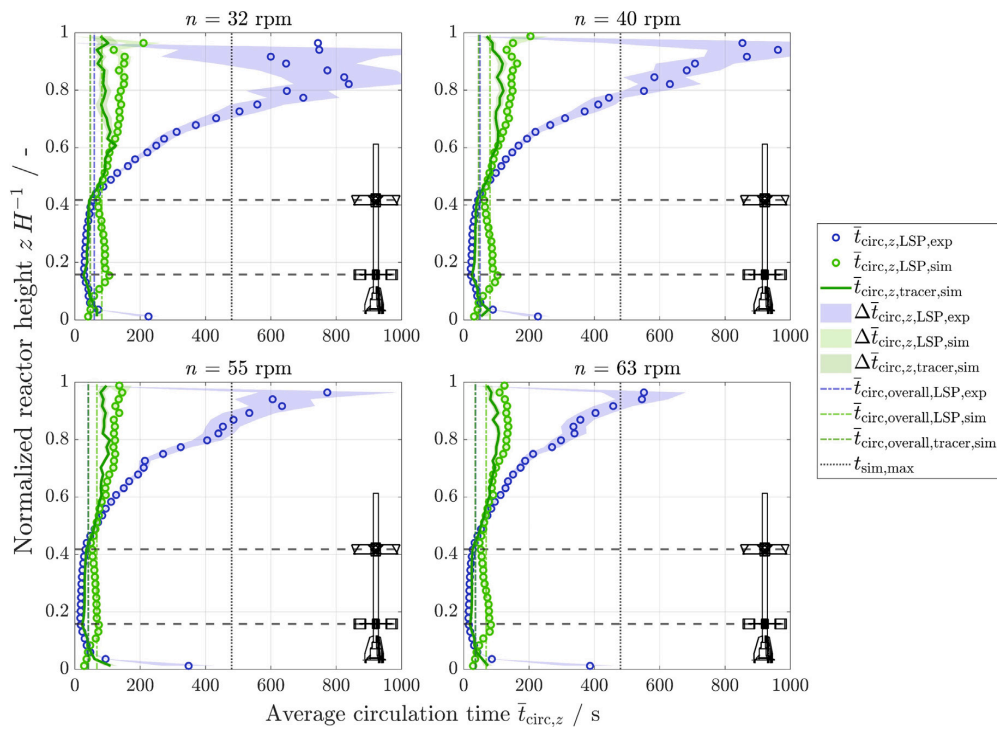


Fig. 9. Average circulation time distribution $\bar{t}_{\text{circ},z}$ as a function of the normalized reactor height z ($H = 4.2\text{ m}$) for experimental LSPs (LSP, exp , blue circles), simulated LSPs (LSP, sim , green circles), and for simulated tracers ($\text{tracer}, \text{sim}$, green line), each for $n \in \{32, 40, 55, 63\}$ rpm. The vertical bin size is 10 cm. The shaded areas depict the uncertainty Δ of the respective dataset, the vertical dotted gray line marks the physical simulation time, and the vertical dash-dotted lines denote the overall circulation time $t_{\text{circ,overall}}$. The gray dashed lines indicate the center position of the RT-6 (lower) and PBT-6 (upper). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3

Summary of maximum traveled distances for each rpm case for all experimental LSP runs (LSP, exp), simulated LSPs (LSP, sim) and simulated, undisturbed tracers ($\text{tracer}, \text{sim}$) for a total of M trials each.

Particle type	Impeller frequency n / rpm			
	32	40	55	63
$\bar{d}_{\text{travel}, LSP, \text{exp}} / \text{m}$ ($M \geq 14$)	381.3 ± 8.6	460.8 ± 17.2	603.0 ± 22.9	661.0 ± 33.2
$\bar{d}_{\text{travel}, LSP, \text{sim}} / \text{m}$ ($M = 98$)	26.0 ± 0.3	30 ± 0.5	38.2 ± 0.7	40.4 ± 0.6
$\bar{d}_{\text{travel}, \text{tracer}, \text{sim}} / \text{m}$ ($M = 98$)	39.2 ± 1.0	45.3 ± 1.3	58.4 ± 1.4	69.8 ± 1.8

the macromixing process within an STR [81], conducted primarily by means of the tracer injection method, for example, measuring the conductivity at a limited number of points [82]. Nonetheless, numerous publications have proved that LSPs may deliver not only the CTD over the reactor height of large tanks, but important data such as general flow structures and hydrodynamic compartments [28,32,76,83,84].

Fig. 9 shows the CTD over the normalized reactor height for the four rpm cases $n \in \{32, 40, 55, 63\}$ rpm with their respective overall weighted average $\bar{t}_{\text{circ,overall}}$ (Table 4). Interestingly, the four undisturbed tracer trends (green line) differ only marginally from each other, which is reflected in the small overall average circulation time difference of ≈ 12 s between the lowest and highest impeller frequency. The lowest circulation time is at the height of the RT-6, which marks the turnover point in the reactor [68]. Both trends of the tracers and the experimental LSPs (blue dots) have similar low circulation times between the impellers and similarly increasing toward the reactor bottom. Even so, their values split at a height of $z/H^{-1} \approx 0.5$, from which the LSP, exp circulation times increase by up to 30 times higher values, compared

to the high turbulent zone. At a normalized height of $z/H^{-1} \approx 0.8$, the trend shows unsteadiness at all impeller frequencies, with values increasing more slowly. The high uncertainties are attributed to the sparse number of data points in the upper third of the STR (Fig. 8). The trends of the simulated LSPs (green dots) demonstrate a peak at the height of the RT-6, with dropping values toward the PBT-6, and again increasing values above the latter toward the top of the reactor. Hence, the peak at the RT-6 denotes a boundary, which they cross less often. The lowest circulation times are in the dish bottom of the STR, which might be due to their clustering behavior and short turning circles. Surprisingly, their distinctive peaks are at the same heights, where the other two types of particles show either their lowest values ($z/H^{-1} \approx 0.15$) or start to increase sharply ($z/H^{-1} \approx 0.45$). In summary, the CTDs and their respective overall average values are similar to those of previous work in the same reactor, which was equipped with three Elephant Ear impellers [32].

Small circulation times indicate small and fast turning circles, also in the form of vortices, and thus are reflected as well-mixed zones. Vertical hydrodynamic compartments can be determined at heights, where $\bar{t}_{\text{circ},z}$ increases more rapidly toward higher values, just below RT-6 at $z/H^{-1} = 0.15$ and above PBT-6 between $0.45 < z/H^{-1} < 0.55$. Notably, the LSP, sim and the CTD of the tracers do not increase as rapidly toward the reactor top as the LSP, exp . The main reason is most likely due to the physical simulation time of $t_{\text{sim,max}} = 480$ s (vertical dotted gray line) and the resulting shorter maximum traveled distance compared to the experiments (Table 3). Hence, their CTD must be interpreted with caution with regards to their amount of data and the length of experiments, as was explained by Mann et al. and Day [84,85].

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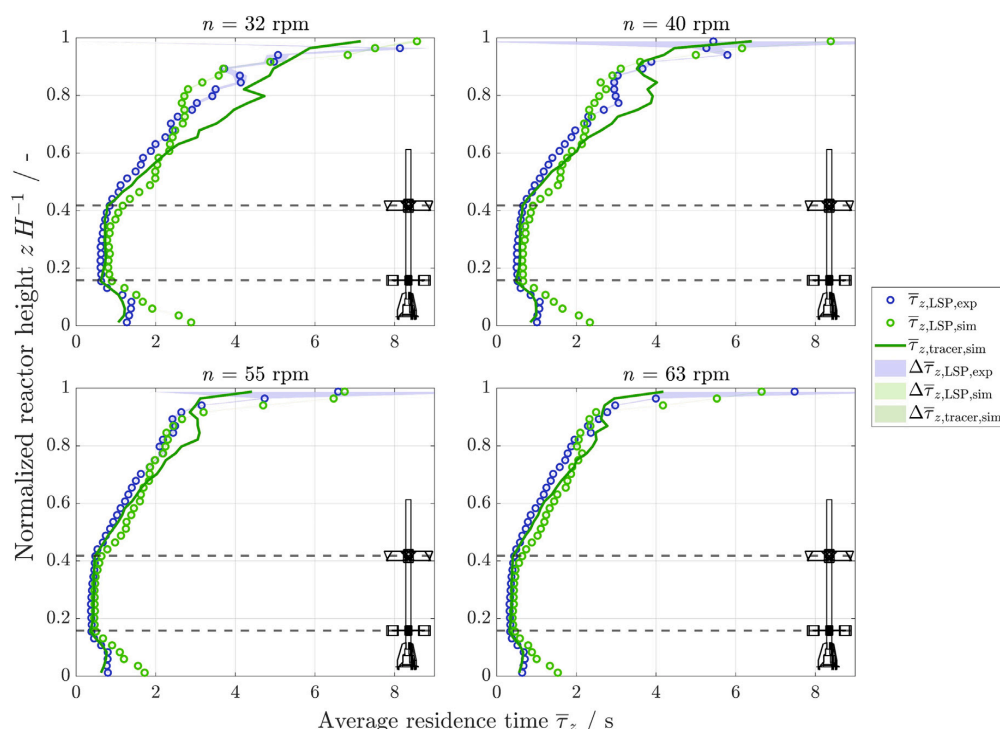


Fig. 10. Average residence time distribution $\bar{\tau}_z$ as a function of the normalized reactor height z ($H = 4.2$ m) for experimental LSPs (LSP,exp , blue circles), simulated LSPs (LSP,sim , green circles), and for simulated tracers ($tracer,sim$, green line), each for $n \in \{32, 40, 55, 63\}$ rpm. The vertical bin size is 10 cm. The shaded areas depict the uncertainty Δ of the respective dataset, and the vertical dash-dotted lines denote the overall average residence time $\tau_{overall}$. The gray dashed lines indicate the center position of the RT-6 (lower) and PBT-6 (upper). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

It appears that the maximum circulation times that occur for both LSP,sim and LSP,exp are a fourth of the respective maximum trial length. The maximum traveled distance is approximately 15 times higher for LSP,exp .

3.5. Residence time distribution

The average hydraulic residence time distribution (RTD) provides information about the tracer's and LSP's average duration in the respective reactor slice j , each with a height of 0.1 m, based on their measured absolute average axial velocity. With regard to the investigation of population heterogeneity within a bioreactor and the classification of cell productivity regimes, this information is vital to conceptualize, manufacture, and evaluate a suitable scale-down reactor design. Laboratory-scale reactors are able to simulate large-scale conditions to understand the interplay between the stress on the microorganism and the resulting product limitations [5,15,19,86,87].

Fig. 10 shows the average residence time distribution over the normalized reactor height for all impeller frequencies $n \in \{32, 40, 55, 63\}$ rpm. The tracer particles' (green line) shortest average residence times are at the height of the RT-6 (lower gray dashed line) with slightly increasing values toward the PBT-6. Below and above the impellers, the residence times are two-fold or even 10-fold higher, respectively. At all impeller frequencies, the trend shows an anomaly between $0.7 < z/H < 0.9$, which is barely seen for LSP,sim (green dots), but fairly so for LSP,exp (blue dots). However, a similar behavior is observed at the average circulation times of LSP,exp at the same height (Fig. 9). Most likely, two fluid flows converge at this height: the direct upward flow from the baffles, which reflects at the liquid surface back downward, and a counteracting, non-directional short-circuit flow. The RTD of

Table 4

Overview of results of average overall circulation times, overall cumulated residence times, global mixing times, and respective uncertainties.

Time	Particle type	Impeller frequency n / rpm				
		24	32	40	55	63
$\bar{t}_{circ,overall}$ / s	LSP,exp	–	59.4 ± 2.7	49.7 ± 2.1	40.8 ± 1.6	36.4 ± 1.3
	LSP,sim	–	85.0 ± 0.9	82.4 ± 0.8	70.3 ± 0.6	69.5 ± 0.6
	$tracer,sim$	–	48.4 ± 0.7	46.4 ± 0.7	42.2 ± 0.5	35.7 ± 0.4
$\bar{\tau}_{overall}$ / s	LSP,exp	–	81.1 ± 2.5	73.2 ± 7.6	56.9 ± 5.4	52.6 ± 4.0
	LSP,sim	–	99.5 ± 1.0	83.7 ± 0.7	69.1 ± 0.6	63.3 ± 0.5
	$tracer,sim$	–	99.9 ± 0.8	81.2 ± 0.6	59.3 ± 0.4	52.7 ± 0.4
$\theta_{glob,95}$ / s		247 ± 14	191 ± 5	151 ± 9	106 ± 3	102 ± 13

the experimental LSPs is similar to the tracer's, although their values start to differ slightly above the PBT-6 to smaller residence times. Nonetheless, in particular, for increasing impeller frequencies, both show good agreement. On the other hand, although the residence times of the LSP,sim between the impellers are alike the other two particle types, they show increasing values toward the bottom of the reactor and discontinuous trends toward the top. In summary, all three trends show a distinctive region with comparatively low residence times between $0.15 < z/H < 0.45$ and rapidly increasing values below and above, respectively, which marks vertical hydrodynamic compartment barriers. Compared to a previous study in the same reactor, equipped with three Elephant Ear impellers, the RTD values, as well as the overall cumulative residence times, are similar (Table 4), in particular for low impeller frequencies [32].

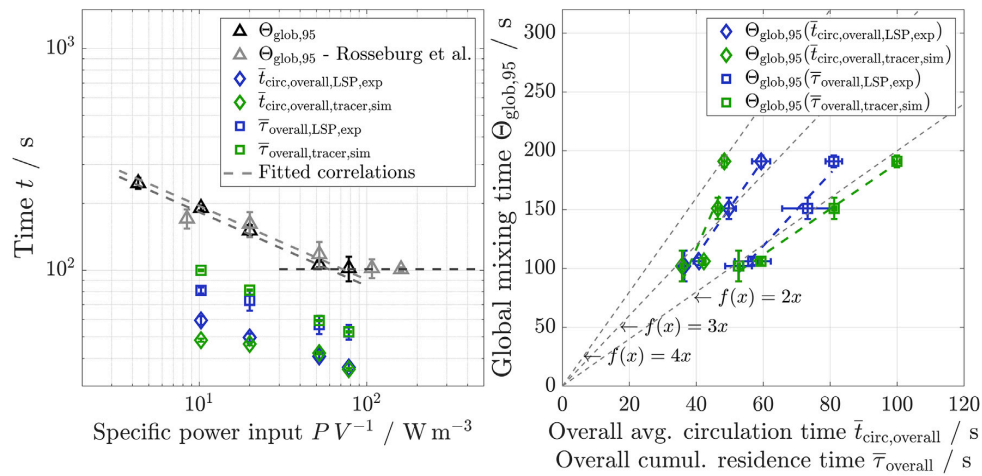


Fig. 11. Left: Measured global mixing times $\Theta_{\text{glob},95}$ (black triangles), global mixing times from Rosseburg et al. in the same reactor setup (gray triangles) [43], overall average circulation times $\bar{t}_{\text{circ,overall}}$ (diamonds), and overall cumulated residence times $\bar{\tau}_{\text{overall}}$ (squares) as a function of the specific power input $P V^{-1}$. For the last two, values for experimental LSPs (LSP, exp , blue) and for simulated tracer (tracer, sim , green) are shown. Dashed lines are literature correlations. Right: Global mixing time as a function of overall average circulation time and overall cumulated residence time with a respective linear correlation for experimental LSPs (blue) and tracer (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 5

Overview of empirical mixing time correlations from literature for multi-staged impellers in an unaerated STR.

Correlation	Comment	Author
$\Theta_{\text{glob}} = B \cdot P_0^{-1/3} \cdot \left(\frac{n}{60}\right)^{-1} \cdot \left(\frac{H}{d_{\text{STR}}}\right)^{2.43}$	B: fitting const.; here: 2.1	Cooke et al. [94]
$\Theta_{\text{glob}} = B \cdot \left(\frac{d_{\text{STR}}}{d_{\text{imp}}}\right)^{1/3} \cdot \left(\frac{H}{d_{\text{STR}}}\right)^{2/3} \cdot (N_{\text{imp}} + 1)^{2/3}$	B: fitting const.; here: 16.5	Sieblist et al. [95]
$n \cdot \Theta_{\text{glob},95} = 2.3 \cdot \exp\left(0.68 \cdot \frac{d_{\text{STR}}}{d_{\text{imp}}} + 0.83 \cdot N_{\text{imp}}\right)$		Vasconcelos et al. [96]

3.6. Mixing times

In a fed-batch cultivation of animal cells, nutrients are added according to precise feeding strategies after being metabolized to achieve optimal product yield and titer. [88]. Process intensification strategies, such as perfusion processes, result in very high cell densities, yet require a high degree of homogeneity throughout the reactor [8]. Therefore, mixing time is a key parameter as it is critical for cell growth and, eventually, for the quality and quantity of the product. Moreover, it is a core characterization parameter for a bioreactor and serves as both a modeling parameter and a validation measure for numerical simulations, in particular for scale-up and scale-down strategies [60,82,89–92]. In this study, the decoloration method by means of utilizing bromothymol blue (BTB) is evaluated according to Paul et al. [74] at a 95% mixedness. The contrasting color change of the BTB and the resulting logarithmic variance of the normalized gray scale enables a detailed temporal progress of either the STR as an entirety (global mixing time $\Theta_{\text{glob},95}$) or highly resolved (local mixing time distribution $\Theta_{\text{loc},95}$) [93] for a compartment localization. The basic methodology of the latter is elucidated in Fitschen et al. [59].

In Fig. 11 (left), the global mixing times measured $\Theta_{\text{glob},95}$ from this study (black triangles, Table 4) and from a previous study (gray triangles) by Rosseburg et al. [43] are plotted as a function of the specific power input. The latter were obtained in the same reactor ($V = 12.0 \text{ m}^3$, $H d_{\text{STR}}^{-1} = 2.0$) by means of the decoloration method with phenolphthalein. All values agree well with the correlations given by Cooke et al. [94] (light gray dashed line) and Sieblist et al. [95] (dark gray dashed line), if taking into account the fitting constants $B = 2.1$ and $B = 16.5$, respectively (Table 5). The horizontal dashed line denotes a range in which, according to Zlokarnik, the mixing time is independent of the stirrer speed [57]. This smallest possible mixing

time seems to be $\Theta_{\text{glob},95} \approx 100 \text{ s}$. Furthermore, global mixing times from a recent study on hydrodynamic stress in the identical reactor fit in the same correlation [13], as well as the results from Bernemann et al. [90] (not shown here). In addition, overall average circulation times (diamonds) and overall cumulative residence times (squares) are plotted in the same graph for the experimental LSPs (LSP, exp , blue) and for the tracer particles (tracer, sim , green) as a reference point. Their trends demonstrate similar behavior. With an increasing power input, their values align within a comparatively low uncertainty. The primary reason for the discrepancy in low $P V^{-1}$ values is mainly due to their difference in recording length (Chap. 3.4) and a combination of limited data points for LSP, exp and elevated residence times within the fluid reflection zone in the top of the reactor (Chap. 3.5), respectively.

Fig. 11 (right) shows the overall average circulation times (diamonds) and the overall cumulated residence times (squares) in the context of their respective global mixing time with a linear regression for the experimental LSPs (LSP, exp , blue) and for the tracer particles (LSP, sim , green). The circulation time ratios are shown to be $2.6 \cdot \bar{t}_{\text{circ}} < \Theta_{\text{glob},95} < 4.0 \cdot \bar{t}_{\text{circ}}$, depending on the particle type, where the tracer data have a higher ratio in part than the experimental data, due to their limited physical time in the reactor. The ratio of the overall cumulated residence time is for both particle types approximately between 2 and 2.4. In particular, in the case of circulation times, previous studies report of $3 \cdot \bar{t}_{\text{circ}} < \Theta_{\text{glob},95} < 5 \cdot \bar{t}_{\text{circ}}$ with conductivity measurements and a single-staged RT-6 ($H d_{\text{STR}}^{-1} = 1.0$) [60,97–99]. The lowest ratio is presented by Bisgaard et al. with $\Theta_{\text{glob},95} = 2.2 \cdot \bar{t}_{\text{circ}}$ [76]. Another work, which exclusively uses three axial pumping impellers (Elephant Ear, ABEC design) on industrial scale with a $H d_{\text{STR}}^{-1} = 2.1$ reports of $1.1 \cdot \bar{t}_{\text{circ}} < \Theta_{\text{glob},95} < 1.5 \cdot \bar{t}_{\text{circ}}$, similar to a model approach by van de Vusse [100]. Hence, the results of this study coincide well with the results of the literature, considering an RT-6 and a PBT-6 with $H d_{\text{imp}}^{-1} = 2.1$.

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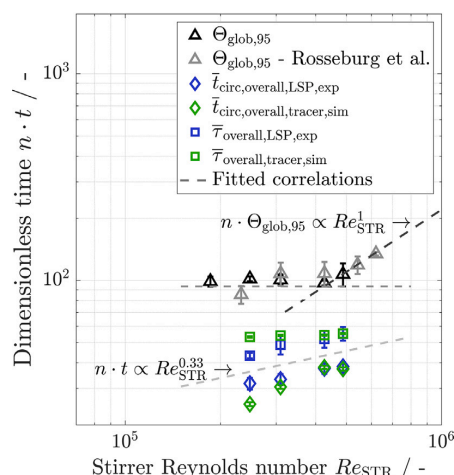


Fig. 12. Dimensionless time $n \cdot t$ as a function of the stirrer Reynolds number Re_{STR} with $t = \Theta_{glob,95}$ as dimensionless mixing time (black triangles), $t = \bar{t}_{circ,overall}$ as dimensionless circulation time (diamonds), and $t = \bar{\tau}_{overall}$ as dimensionless residence time (squares). For the last two, the experimental LSPs (LSP,exp , blue) and tracer ($tracer,sim$, green) are shown. Linear correlations are depicted as dashed lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Aforesaid results can be displayed via dimensionless time ($n \cdot t$) as a function of the stirrer Reynolds number Re_{STR} [57], together with widely used correlations found in the literature (Table 5). Fig. 12 indicates all the experimental results of the mixing times (triangles), overall average circulation times (diamonds), and overall cumulated residence times (squares). As described by Zlokarnik [57], the mixing time results at given Reynolds numbers in this study can be subdivided into two ranges. The first range is $n \cdot \Theta_{glob,95} = \text{const.}$, below the Reynolds numbers $Re_{STR} < 4.3 \cdot 10^5$, which coincides with the correlation by Vasconcelos et al. [96] (Table 5). This range is also described and analyzed in other publications [95,101–103]. With an increasing impeller frequency, and thus higher Reynolds numbers $Re_{STR} > 4.3 \cdot 10^5$, the correlation changes to $n \cdot \Theta_{glob,95} \propto Re_{STR}^1$ in the second range, also described by Rosseburg et al. [43] and Gabelle et al. [104]. Any other mixing time correlation found describes either a setup with only one single impeller type or an STR with mostly $H d_{STR}^{-1} = 1$ [74,102,105,106]. Interestingly, the experimental overall average circulation time and overall cumulated residence time do not obey this correlation. They indicate $n \cdot \Theta_{glob,95} \propto Re_{STR}^{0.33}$, at least with the limited number of data points.

A more detailed analysis is demonstrated in Fig. 13, which shows the relative local mixing time distribution Θ^* throughout the reactor at four impeller frequencies $n \in \{32, 40, 55, 63\}$ rpm. According to Eq. (32), the calculated local mixing time in a 2×2 pixel array is normalized by the respective global mixing time $\Theta_{glob,95}$. From a qualitative point of view, three compartments are formed in the STR: one in the bottom of the reactor, below the RT-6 at $z H^{-1} \approx 0.15$, the second between the RT-6 and the vertical center of the reactor at $z H^{-1} \approx \{0.54, 0.49, 0.44, 0.49\}$, and a third compartment in the top region. An overall similarity of the relative mixing time distributions reveals that prevailing compartments are almost independent of the impeller frequency, as was also reported in an earlier work in a 3L STR [107]. For the first time, detailed hydrodynamic compartments on industrial scale are illustrated based on mixing time experiments, and demonstrate a high agreement with the aforementioned LSP results (Chap. 3.2, 3.4, 3.5), and furthermore, a number of foregoing experiments [108] and numerical simulations regarding this topic [63,109]. However, evaluating mixing times in an

industrial-scale acrylic glass STR contains challenges and causes errors. Although sufficient and homogeneous background lighting is provided, one wide-angle lens at the designated height and distance creates distortion, in particular toward the top of the reactor. For cylinder imaging evaluations, it is recommended to work with refraction compensation [110], which can be achieved by implementing containers outside [34,90], albeit not realistic for this reactor size. Moreover, outer obstacles or moving objects, such as cross beams, tank nozzles, acrylic glass flanges, the stainless steel dished tank bottom, and impellers, hinder an evenly illuminated view of the inside. Nevertheless, suitable post-processing steps yield a spatio-temporal resolved 2D map that highlights coherent structures primarily influenced by mixing.

3.7. Lagrangian regime analysis

Substrate gradients in industrial bioreactors affect the cell's biokinetics and metabolic dynamics, leading to population heterogeneity (changes in the metabolome, proteome and transcriptome) and eventually to a decline in growth and production [5,111,112]. Therefore, life-line analysis has become a valuable tool for developing scale-down simulator concepts or even microfluidic chips in order to mimic large scale conditions on a laboratory scale [19,113,114]. The Euler–Lagrange concept was introduced by Lapin et al. [30] and has gained attention in recent years [31]. Haringa et al. presented three Lagrangian analysis methods to interpret a particle's trajectory, of which this study applies the *Lagrangian regime analysis* [115,116].

The discretization of the Lagrangian trajectories from all three particle types provides the residence times in the ascertained single-phase hydrodynamic compartments τ_{regime} , which is depicted in Fig. 14, exemplary for $n = 55$ rpm. The compartment barriers at $z H^{-1} = 0.15$ and $z H^{-1} = 0.44$ are set based on the results in the previous chapters, the CTD, RTD, and local mixing time distribution. Residence times in the upper (red), center (green), and lower compartments (blue) are shown with their probability density. It is seen that LSP,exp (upper) and LSP,sim (center) show a contrasting probability of residence times until $\tau_{regime} \approx 20$ s for the upper and lower compartment. This behavior can be explained by their different probability of presence over the reactor height (Fig. 8). In addition to that, both trends are generally in good agreement with the tracer results ($tracer,sim$, lower). However, a substantial condition might arise at probabilities greater than $\tau_{regime} > 117$ s (vertical dashed line) in the upper compartment, which is a theoretically calculated limit for a Chinese Hamster Ovary (CHO) cell to be oxygen-deprived [32], especially at high biomass concentrations. Associated studies with CHO cells were conducted by Gaugler et al. and Anane et al. [19,25]. When exposed to dissolved oxygen gradients, they report a more rapid decline in viable cell count and an increase in lactate concentrations.

In summary, the findings indicate a 0.1%, 2.3%, and 1.3% probability that the LSP,exp , the LSP,sim , or $tracer,sim$, respectively, will remain longer in the upper compartment than the theoretical limit allows. With a lower limit of oxygen deprivation of 90 s, the probabilities increase to 0.4%, 4.2%, and 1.7%, respectively. The LSP,exp slightly underrepresented the tracers' probability, notwithstanding their longer maximum travel distance during a trial, owed to their decreased probability of presence in the top of the STR.

3.8. Stokes number estimations

Lagrangian Sensor Particles are large, nearly neutrally-buoyant particles that move with the turbulent surrounding carrier flow in a characteristic way. Their size is three orders of magnitude larger than the Kolmogorov length scale and approximately one order of magnitude larger than the Taylor scale. Due to their high inertia, the resulting drag causes a relevant reaction time lag to changes in fluid velocity [50]. In the context of flow-following characteristics and for classification benefits, this study offers various methodologies to estimate the Stokes

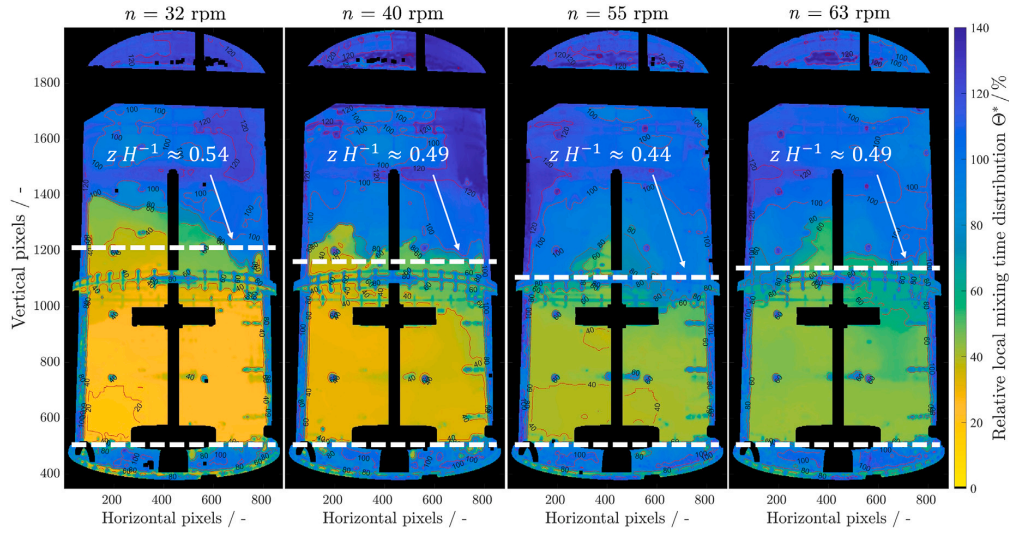


Fig. 13. Comparison of relative local mixing time distributions Θ^* (local mixing time $\Theta_{loc,95}$ in a 2×2 pixel array normalized by the respective global mixing time $\Theta_{glob,95}$) at four impeller frequencies $n \in \{32, 40, 55, 63\}$ rpm. The global mixing times are $\Theta_{glob,95} \in \{191 \pm 5, 151 \pm 9, 106 \pm 3, 102 \pm 13\}$ s, respectively. The injection point (IPO) is marked in Fig. 1. Due to the recording distance of the camera, the camera lens used, and the resulting image distortion, pixels are specified instead of distances.

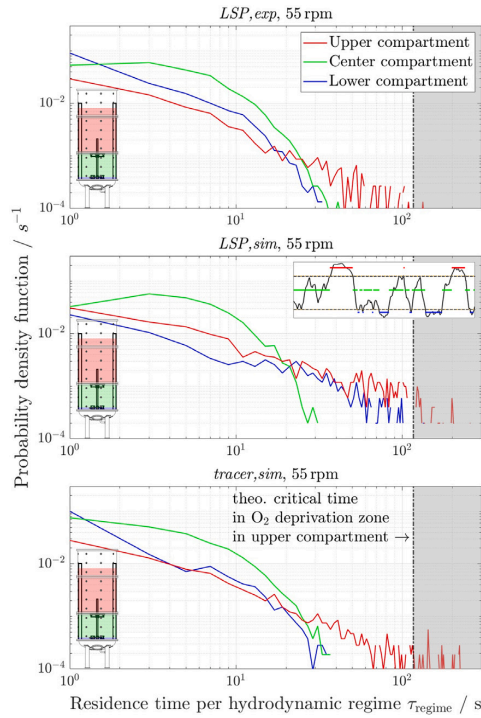


Fig. 14. Probability density function of the residence time per hydrodynamic regime τ_{regime} for the upper (red), center (green) and lower (blue) regime for the experimental LSPs (LSP,exp), simulated LSPs (LSP,sim) and tracer ($tracer,sim$) at $n = 55$ rpm. Compartment barriers are taken from CTD, RTD and relative local mixing time distributions at $z H^{-1} = 0.15$ and $z H^{-1} = 0.44$. The vertical dashed line and gray area indicates a theoretical time limit at 117 s for a CHO cell to be oxygen limited. Inset: Exemplary trajectory discretization with the respective compartment color. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

number; however, it does not adequately predict the dynamics of LSPs of this magnitude [61,117]. Generally, this research outlines three approaches for Stokes number estimations based on the underlying data and the assumptions made. The results are listed in Table 6.

The first approach utilizes only the experimental axial velocity data obtained to calculate the slip velocity in Re_p and the resulting drag coefficient to estimate τ_p (Eq. (8)). The large eddy turnover times τ_t are calculated with Eqs. (12)–(14) so that the calculated Stokes numbers are approximately two orders of magnitude apart from each other (Table 6, upper part). Obviously, the used LSPs with a diameter of $d_p = 40$ mm demonstrate a minor flow-following behavior on the micro-scale; however, a Stokes number of St (meso-scale) ≈ 0.2 indicates that they are indeed able to detect compartment barriers and heterogeneities on the meso-scale, as the previous chapters impose, in particular concerning the local mixing time distribution. On the macro-scale, the LSPs show similar results to the simulated undisturbed fluid flow tracers regarding the overall average circulation time (Fig. 11). This similarity is due to the comparatively short physical time in the STR of the latter, which explains the low St (macro-scale) ≈ 0.002 . Hence, this approach provides a fair assessment of the LSPs utilized by applying only experimental data.

The second and third approaches utilize only numerical simulation data and differ in their way of calculating the slip velocity and eventually demonstrating the final data: either as a single stochastic value, which removes the bias and focuses on fluctuations [61] or as a detailed 2D distribution with bin size L representing the mean flow (MF) [32]. In Table 6 (center), the stochastic values are stated. The 2D distributions are shown in Fig. 15, whereupon the respective average values are also indicated in Table 6 (lower part). Both approaches are able to calculate the Stokes number and, in addition, a theoretical maximum particle diameter, which would actually be able to follow the flow with the given fluid velocity data in the STR [34]. In that case, a Stokes number of $St = 0.1$ is assumed [53], according to which the term $(c_D Re_p)/24$ approaches unity, since now Stokes drag applies [118]. The theoretical particle diameter is calculated as

$$d_{p,th} = \sqrt{\frac{18 St \eta L}{\rho_p u}}. \quad (33)$$

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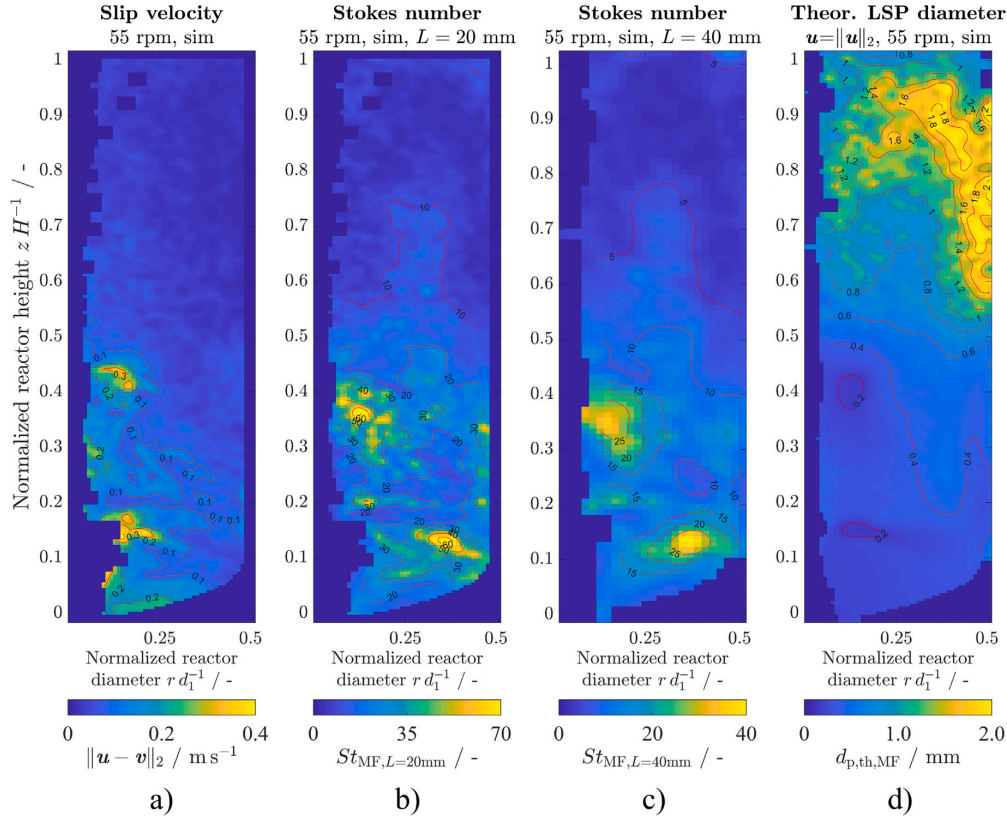


Fig. 15. Numerical data results depicted as a 2D distribution in a predefined bin size L representing the mean flow (MF) for an impeller frequency of $n = 55$ rpm for both simulated particle types *LSP,sim* and *tracer,sim*. (a) The slip velocity distribution calculated by means of the second euclidean norm with $L = 20$ mm. (b) The consequent Stokes number distribution St_{MF} with $L = 20$ mm. (c) The Stokes number distribution with a doubled $L = 40$ mm. (d) Theoretical, maximum particle diameter $d_{p,th,MF}$ to comply with the flow-following criterion according to $St = 0.1$. All data is time-, axially- and azimuthally-averaged.

Table 6

Overview of results of Stokes number estimations on different scales, approaches and respective equations, calculated slip velocities and estimated theoretical particle sizes necessary to be flow-following in the STR.

		Equations	Impeller frequency n / rpm			
			32	40	55	63
experi- mental	St (micro-scale) / -	(8), (14)	29.4	34.2	41.3	42.9
	St (meso-scale) / -	(8), (12)	≈ 0.20			
	St (macro-scale) / -	(8), (13)	≈ 0.002			
LB LES	$V_{slip,stoch}$ / $\text{m s}^{-1} \cdot (10^{-2})$	(15)	8.6	10.4	13.5	17.0
	St_{stoch} / -	$L = 20 \text{ mm}$, (15), (16)	1.2	1.4	1.9	2.0
	$d_{p,th,stoch}$ / mm	(33), $u = u_{stoch}$	1.5	1.3	1.0	0.8
LB LES	$\ u - v\ _2$ / $\text{m s}^{-1} \cdot (10^{-2})$		5.4	6.4	7.9	8.9
	St_{MF} / -	$L = 20 \text{ mm}$, (17)	13.9	13.9	15.6	16.1
	$d_{p,th,MF}$ / mm	(33), $u = \ u\ _2$	0.9	0.8	0.7	0.7

which again delivers either a single stochastic value $d_{p,th,stoch}$, or a 2D distribution based on the mean flow grid of $L = 20$ mm and its average value $\bar{d}_{p,th,MF}$.

As shown in Table 6, the stochastic Stokes number St_{stoch} is approximately one order of magnitude underestimated compared to the average Stokes number of the mean flow $\bar{St}_{MF}$ of the 2D distribution. The theoretical maximum particle sizes are in good agreement, particularly with increasing impeller speed.

The 2D distributions in Fig. 15 yield a detailed insight into the hydrodynamics of the STR. The highest slip velocity and, thus, the highest Stokes numbers are found in the vicinity of the impellers. It should be mentioned that the size of the Stokes number obviously scales with the chosen size of the characteristic length scale L in τ_f , which is chosen as $L = 20$ mm (Fig. 15 (b)) and $L = 40$ mm (Fig. 15 (c)) for comparison reasons. Interestingly, the estimated experimental Stokes number on the micro-scale St (micro-scale) = 41.3 (Table 6) is in a similar range as the Stokes number of the *LSP,sim* in the 2D distribution (Fig. 15 (b)) near the impellers, especially if the Stokes number proposed by Rautenbach et al. with the center of mass shift factor of $r^* = 0.79$ is incorporated [33]. Moreover, the theoretical particle sizes to fulfill the Stokes criterion are the lowest in the vicinity of the impellers with $d_{p,th,MF} \approx 0.2$ mm and increase up to 2.0 mm with increasing distance. The bulk phase would be covered with an LSP size of approximately 1 mm, in order to theoretically follow the flow on a micro-scale, based on the characteristic length of the mean flow of $L_{MF} = 20$ mm. Interestingly, in a previous publication, which utilized 4D-Particle Tracking Velocimetry and accompanying LB LES in a 3L STR, similar values were predicted for the industrial scale for a similar specific power input [34].

4. Conclusions

This study represents an analysis of experimentally and numerically derived particle trajectories to hydrodynamically characterize

a single-phase industrial bioreactor for heterogeneities and compartments occurring at different impeller frequencies. The stirred tank reactor (STR) is equipped with a Rushton turbine (RT-6) and a pitched blade turbine (PBT-6) and constitutes an industrial replica with a filling volume of 12.5 m³, made of acrylic glass. Regarding particle trajectories, on the one hand, Lagrangian Sensor Particles (*LSP,exp*), outfitted with a pressure sensor, yield the experimental time-resolved axial height. On the other hand, resolved particle Lattice-Boltzmann large eddy simulations (LB LES) are utilized to emulate those LSPs in the same STR (*LSP,sim*). Furthermore, they provide undisturbed fluid flow data by means of massless tracer particles (*tracer,sim*), which serve as a reference when elucidating the LSP results. The main goal is to prove the technological readiness of LSPs, discuss their size and number in the STR, and provide crucial information about future evaluations.

This work finds that the contrary behavior in the axial probability of presence over the reactor height between *LSP,exp* and *LSP,sim* is most likely due to four main facts. Firstly, the difference in their mass distribution; secondly, their slightly different density; thirdly, the particle number in the reactor; and fourthly, most likely a sufficient but improvable particle-to-grid size ratio. As demonstrated in Rautenbach et al. LSPs with a homogeneous mass distribution, such as used in the simulation for *LSP,sim*, show a decisively higher probability of presence in less turbulent regions and less momentum required to correspond to instantaneous changes in fluid flow [33]. Moreover, the slightly higher density of *LSP,sim* of 0.7 kg m⁻³ provides them with a higher cluster probability in the bottom of the STR, and additionally, an increasing particle collision and perturbation probability of at least 4% due to their high number of particles in the reactor. Due to the high computational demand, the number of particles had to be increased to approximately 250 to reach the same physical trial time (13 h in total per dataset), instead of utilizing only a maximum of three LSPs at the same time for a period of 1 h each (in the case of experiments). Beyond, the particle-to-grid size ratio of 10 might be still too little to accurately calculate the two way coupled fluid–particle forces in the computational boundaries in the lattice structure. Generally, by representing the data density over the reactor height, that information helps understanding the upcoming results.

The aforementioned results on the axial probability of presence in combination with the accompanying sensitivity study outline the axial velocity difference between *LSP,exp* and *LSP,sim*. The latter exhibit higher probabilities for slow velocities, likely due to their comparatively higher probability of presence in the upper reactor zone, which is less turbulent. Furthermore, the high particle volume fraction utilized in the LB LES increases collisions and clustering, attenuates the carrier flow according to the decrease in velocity fluctuations, reduces turbulent kinetic energy by at least 3%, and ultimately leads to a decrease in the LSP's velocity. To avoid these effects, a maximum particle volume fraction of $\approx 10^{-4}$ should not be exceeded for future experiments. Moreover, the homogeneous mass distribution of *LSP,sim* makes them more responsive to horizontal flow changes, resulting in slower axial velocities. However, except for very high velocities, which are obviously characteristic fluid speeds near the impeller blades, both LSP types generally show good agreement.

Furthermore, this work elaborates that the CTDs of *LSP,exp* and *tracer,sim* look alike below the vertical center of the reactor. A distinctive high-turbulent region with small circulation times marks a compartment with two boundaries, below the RT-6 and just above the PBT-6. Above the latter, *LSP,exp* demonstrate 30-fold higher circulation times, owing to their low probability of presence in the upper reactor region and a slight density difference. Since the physical time of the simulated particles is only 480 s, compared to 3600 s for *LSP,exp*, the average circulation times of both LSP types, experimental and simulated, are hardly comparable in this region.

The distributions of the residence times are fairly similar for all particle types, marking the same well-mixed region, and thus three

horizontal compartments with barriers at the same height as evaluated with the CTD.

Consequently, mixing time trials reveal that global mixing times $\Theta_{\text{glob},95}$ fit in the context of literature results that include different correlations. In particular, the ratio of the overall average circulation times $\bar{t}_{\text{circ}}$ of *LSP,exp* and *tracer,sim* agree well with previous publications, as they show $2.6 \cdot \bar{t}_{\text{circ}} < \Theta_{\text{glob},95} < 4.0 \cdot \bar{t}_{\text{circ}}$, depending on the type of particles. The dimensionless residence times $\bar{\tau}_{\text{overall}}$ show $n \cdot \bar{\tau}_{\text{overall}} \propto Re_{\text{STR}}^{0.33}$. The relative local mixing time distribution Θ^* confirms the three compartments, acquired by means of the CTD and RTD at $z H^{-1} \approx 0.15$ and $z H^{-1} \approx 0.50$.

The Lagrangian regime analysis utilizes ascertained compartment barriers to discretize the particle trajectories. It further illustrates the probability of how likely it is for the particles, which are supposed to mimic the lifeline of a Chinese Hamster Ovary (CHO) cell, to be situated in an oxygen-deprived zone in the upper STR compartment. With an assumed oxygen deprivation limit of 90 s, *tracer,sim* shows a probability of 1.7%, while the results for *LSP,exp* (0.4%) and *LSP,sim* (4.2%) are respectively under and overestimated due to their characteristic probabilities of presence.

Multiple approaches are presented for the estimation of the Stokes number St , based on the available data and assumptions, and serve as a point of reference. Solely determined with experimental data, with $St \approx 0.20$ on the meso-scale, and $St \approx 0.002$ on the macro-scale, *LSP,exp* demonstrate sufficient flow-following capability to detect hydrodynamic compartments, as is proven in this study. Regarding the Stokes number on the micro-scale of *LSP,sim*, the average of a 2D distribution and the stochastic value are between $13.9 < \bar{St}_{\text{MF}} < 16.1$ and $1.2 < St_{\text{stoch}} < 2.0$, respectively. Consequently, a theoretical LSP size of $d_{\text{pth}} \approx 1$ mm is calculated, which would satisfy the flow-following requirement of $St = 0.1$ on the micro-scale in the bulk phase of the bioreactor.

Eventually, extending the application of LSPs beyond bioreactors is essential, particularly to evaluate the impact of hydrodynamic heterogeneities on process efficiency and economic performance. Ongoing research within the DFG Collaborative Research Center (CRC 1615 “SMART Reactors for Future Process Engineering”) at the Hamburg University of Technology (TUHH) investigates applications in other industrial processes, such as chemical reactors, focusing on particle-laden flows and sensor particle swarms. While this study provides insights into single-phase bioreactor heterogeneities, applying these findings to multiphase systems requires further research due to added complexities like mass transfer and varying bubble size distributions. Related research with LSPs in the Clean Water Technology Lab (CLEWATEC) at the Helmholtz-Zentrum Dresden-Rossendorf (HZDR) examines hydrodynamics in biogas fermentors, which face poor mixing due to highly complex substrates, low height-to-diameter ratios, and varied agitator designs. These studies aim to optimize efficiency and resource usage, although experimental work during the operation of biogas plants is limited by the risk of process instability and economic losses.

To summarize, this study demonstrates that state-of-the-art LSP technology is an effective tool for investigating large-scale stirred tank reactors. The results showcase the capability of LSPs to detect hydrodynamic heterogeneities in the form of compartments, without requiring optical access. Despite their size, LSPs effectively provide mixing data on the meso- and macro-scale, as corroborated by accompanying LB LES and mixing time studies. For a more comprehensive, time-resolved determination of the LSP locations, resolved particle LB LES simulations will be valuable in guiding future LSP designs.

CRediT authorship contribution statement

Sebastian Hofmann: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ryan Rautenbach:** Writing – review & editing, Writing

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– original draft, Validation, Software, Methodology, Formal analysis, Data curation. **Lukas Buntkiel**: Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Conceptualization. **Isabel Sophie Brouwers**: Methodology, Data curation. **Lena Gaugler**: Writing – review & editing. **Jonas Barczyk**: Writing – review & editing. **Jürgen Fitschen**: Writing – review & editing, Methodology. **Sebastian Reinecke**: Writing – review & editing, Project administration, Funding acquisition. **Marko Hoffmann**: Writing – review & editing, Project administration, Funding acquisition. **Ralf Takors**: Supervision, Project administration, Funding acquisition. **Uwe Hampel**: Supervision, Resources, Project administration, Funding acquisition. **Michael Schlüter**: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used “Writefull’s model” in “Overleaf” in order to improve the readability of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

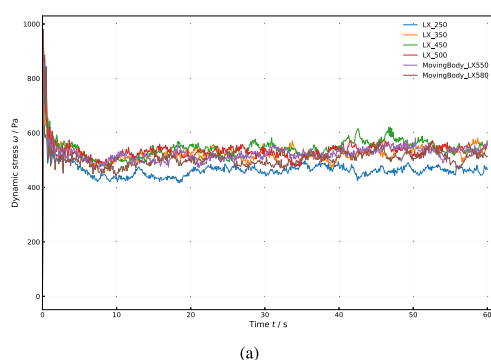
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Grid independence study

See Figs. 16 and 17.



(a)

Appendix B. Sensitivity study

Fig. 18 depicts the probability densities of the three velocity components for both the fluid (upper) and the LSPs (lower), for the two highest volume fractions at 55 rpm. The single-point statistics are normalized by the respective root mean square component calculated from the tracer in an undisturbed flow (dashed line). With regard to the fluid velocity statistics, interestingly, they seem not affected by the presence of LSPs as no statistically significant differences in the trends can be observed as described similarly in Ouellette et al. and Cisse et al. [70,72]. On the other hand, the particle velocity statistics show a clear dampening effect, in particular for higher velocities. This expected result emphasizes the inertia of LSPs, which is similarly shown in previous studies [34,61]. Both particle types and the undisturbed flow tracer demonstrate a non-uniform, asymmetric, spatial distribution of their velocity components, which emphasizes a large-scale anisotropic turbulence behavior in the entirety of the STR. The results of the numerical sensitivity study for all particle volume fractions utilized are summarized in the Table 7.

Table 7
Summary of numerical sensitivity study: Statistical results for all particle volume fractions at $n = 55$ rpm.

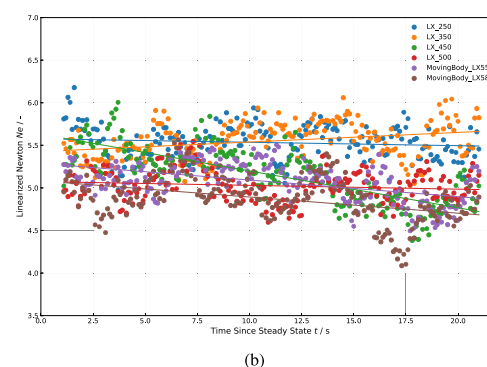
Particle number/–	18	44	92	155	304
Particle volume fraction $\Phi_{LSP,sim} (-10^{-4})/–$	0.48	1.18	2.47	4.16	8.15
τ_c/s	1024	419	200	119	61
$\tau_p \tau_c^{-1} (-10^{-2})/–$	0.10	0.24	0.50	0.84	1.65
PCP/%	0.21	3.00	3.66	3.75	7.33
$u_{rms}^2 (-10^{-2})/m^2 s^{-2}$	5.54	5.14	5.30	5.39	4.72
$u_{\theta,rms}^2 (-10^{-2})/m^2 s^{-2}$	5.37	5.40	4.77	5.27	5.06
$u_{r,rms}^2 (-10^{-2})/m^2 s^{-2}$	4.29	4.14	4.27	4.13	3.85
$k (-10^{-2})/m^2 s^{-2}$	2.51	2.44	2.38	2.44	2.29

Appendix C. Global mixing times

See Fig. 19.

Data availability

Used MATLAB® scripts that are developed in order to evaluate the data can be publicly accessed at the [Gitlab project](#). Raw data and results are publicly accessible in the [DaRUS repository](#). Please contact Sebastian Hofmann, Ryan Rautenbach, or Lukas Buntkiel for further information.



(b)

Fig. 16. Plot (a) showing the dynamic stress over time related to the moving body with an RT-6 and a PBT-6 configuration at different lattice densities and plot (b) of the linearized Power number over time since steady state.

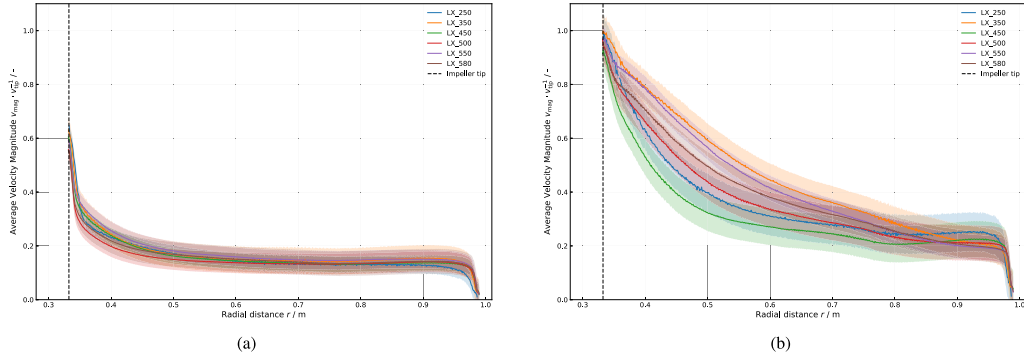


Fig. 17. Plot (a) showing the azimuthal velocity magnitude in the plane of the pitched blade over the radial distance from tip to reactor wall at different lattice densities and plot (b) of the azimuthal velocity magnitude in the plane of the Rushton impeller over the radial distance from tip to reactor wall at different lattice densities.

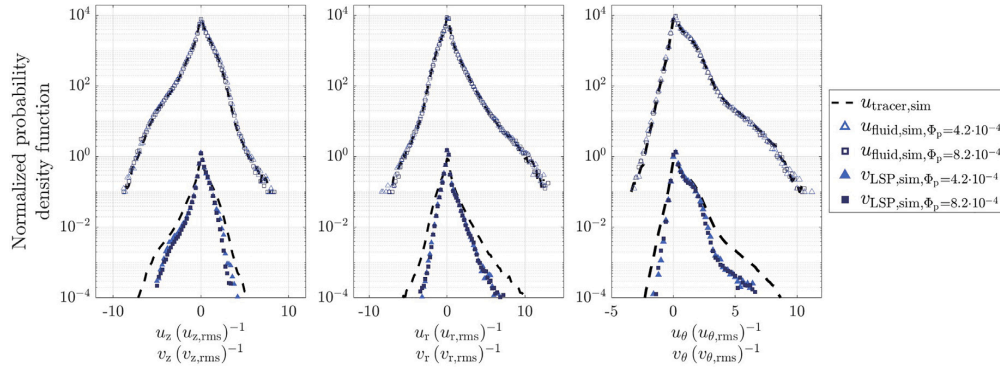


Fig. 18. Normalized probability density of the simulated Lagrangian fluid velocities u (upper) and simulated Lagrangian LSP velocities v (lower), subdivided in cylindrical coordinates z (height), r (radial) and θ (azimuthal) for $n = 55$ rpm for the sensitivity study. Both particle types are simultaneous in the STR. PDFs are offset for clarity. All velocities are normalized by their respective rms value from a separate undisturbed tracer simulation (dashed line).

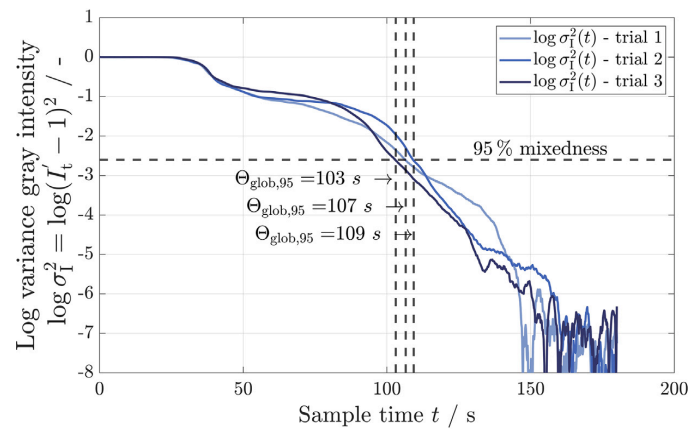


Fig. 19. Logarithmic variance of the normalized gray scale intensity $\log \sigma_1^2$ as a function of the sample time t for three trials at 55 rpm. Resulting time $\Theta_{\text{glob},95}$ reveals the global mixing time at a 95% mixedness [74].

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Chapter 7

Conclusion and outlook

Understanding the complex interplay between fluid dynamics and biological responses is essential for improving the performance and reliability of large-scale bioprocesses. In the context of industrial-scale stirred tank reactors (STR), mixing heterogeneities and the resulting population heterogeneities remain a fundamental challenge. These heterogeneities, both temporal and spatial, influence the environmental conditions that individual cells experience during a cultivation. Such conditions affect cellular metabolism, productivity, and ultimately the quality and quantity of products such as monoclonal antibodies or vaccines. As pharmaceutical bioprocesses evolve toward greater complexity and stricter regulatory frameworks, the demand for robust monitoring and scale-down strategies continues to grow.

Final remarks on the contributions of this thesis

This thesis addresses these challenges by providing a comprehensive theoretical foundation, integrated with three complementary and progressively advancing research studies. The core aim throughout this work is to identify, quantify, and interpret spatiotemporal heterogeneities using inertial particles, such as Lagrangian Sensor Particles (LSP). These mobile, encapsulated, free-floating sensor particles are employed as *in situ* probes to approximate cell trajectories, so called "lifelines", under turbulent flow conditions in STRs of different sizes. Particular emphasis is placed on coupling experimental efforts with Lattice-Boltzmann large eddy simulations (LB LES), which offer a powerful framework for comparison with experimental observations. These simulations are validated using experimental measurements, and thus serve not only as predictive tools but also as a means to gain deeper insight into fluid flow structures that are otherwise difficult to access. Furthermore, LSPs have been tested and evaluated for their ability to detect and resolve hydrodynamic compartments. Their behavior is analyzed in relation to the flow structures they encounter, with particular attention to their

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size-dependent inertial properties on both the laboratory and industrial scale. Ultimately, the results demonstrate their potential to provide critical experimental data for scale-down models.

To conclude this work, the fundamental research questions RQ1, RQ2, and RQ3, as stated in Chapter 3 are revisited, and a brief summary of the key findings of each publication with respect to these questions is provided.

Research questions revisited

RQ1: To what extent can state-of-the-art Lagrangian Sensor Particles represent cell lifelines, and how does their inertial behavior influence this capability?

RQ1.1: Based on Lagrangian particle tracking evaluations in a 3 L STR, supported by numerical simulations, the micro-scale flow-following capability of current state-of-the-art LSPs is found to be insufficient, particularly in turbulent zones in the vicinity of the impellers. However, valuable evaluation metrics are introduced, including Lagrangian single-point statistics and a spatially resolved Stokes number analysis, which aid in understanding inertial particle dynamics. Both experimental and simulated results suggest that miniaturization is essential for accurately mimicking the lifelines of cells. It is also demonstrated that numerical simulations are a critical tool for resolving fluid flow structures and comparing these data with inertial particle trajectories.

RQ1.2: The predictions from the first publication are confirmed: LSPs are unable to accurately represent cell lifelines on industrial scale. Estimated micro-scale Stokes numbers are several orders of magnitude too high to ensure sufficient flow-following behavior. Nevertheless, LSPs exhibit acceptably low Stokes numbers on the macro-scale, enabling them to follow the large-scale flow closely enough to characterize macroscopic hydrodynamics. This is demonstrated by circulation time distributions, which are consistent with flow number correlations and global mixing time measurements. Therefore, while their inertial limitations prevent accurate lifeline representation on the micro-scale, their flow-following capability is adequate for capturing bulk flow phenomena.

RQ1.3: Based on multiple calculation approaches including results from numerical simulations, it is again shown that the estimated micro-scale Stokes numbers for LSPs are far too high to achieve reliable flow-following behavior. However, consistent with findings in the second publication, critical flow characteristics such as circulation times, residence times, and mixing time scaling laws can still be effectively resolved on meso- and macro-scale

using experimental LSPs. As emphasized in the first publication, complementary numerical simulations remain essential to provide massless tracer data, which serve as a benchmark for interpreting LSP measurements. Comparisons between experimental LSP trajectories and massless tracer simulations reveal surprisingly strong agreement in terms of averaged Eulerian data, including axial velocity profiles, circulation times in the center of the reactor, residence times, and overall hydrodynamic regimes.

RQ2: How reliably can accompanying Lattice-Boltzmann large eddy simulations capture the dynamics of inertial particles in bioreactors across scales, and what are their limits?

RQ2.1: The LB LES captures the inertial particle behavior in good agreement with the experimental results. However, simulated inertial particle trajectories exhibit noticeable deviations from experimental observations, especially for the larger alginate beads and in regions of velocity exceeding the tip speed. These discrepancies are most likely caused by numerical limitations such as interpolation errors due to the chosen particle-to-grid-size ratio. Since those larger particles cannot be treated as "point-like", the sub-grid scale model used between lattice nodes mismatches accurate fluid-particle interaction. To treat such particles as "resolved", a significantly finer lattice would be required, additionally necessitating a mesh study. Nonetheless, the reliability of the simulation is high for the smaller inertial particles used. Together, these data are critical for understanding the general dynamics of particles.

RQ2.3: In the industrial setting, simulated LSP data align largely with experimental results for axial velocity and residence time distributions. However, discrepancies remain in the probability of presence and circulation time distributions. These differences arise from inherent physical and numerical limitations, including variations in mass distribution, the number of particles present in the STR, and the chosen particle-to-grid-size ratio. Despite these limitations, the simulation largely reproduces inertial dynamics and, most importantly, provides for the first time a spatially resolved Stokes number grid throughout the reactor and valuable data for a stochastic evaluation.

RQ3: What characteristic flow structures and heterogeneities can be identified through Lagrangian lifeline analysis, and how can these insights support the understanding of industrial-scale bioreactors?

RQ3.1: Eulerian and Lagrangian evaluations reveal overall good agreement between experimental and numerical velocity flow results, highlighting regions of high velocities

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near the impellers and low velocities between both impellers and in the upper region of the STR. A similar agreement is observed for the particle Reynolds number, which serves as the basis for subsequent Stokes number calculations. Although the particle sizes used differ by a factor of four and the resulting spatially resolved Stokes numbers differ by an order of magnitude, both experimental and simulated data still reflect consistent macro-scale flow structures, independent of their inertial behavior.

RQ3.2: The characteristic axial-pumping behavior of the three-staged Elephant Ear impeller configuration is successfully identified using LSPs. Most notably, LSP lifelines reveal three vertically stacked compartments with distinct boundaries, whose positions remain largely unaffected by changes in impeller frequency. Moreover, for the first time, the STR is characterized utilizing estimated dimensionless flow numbers, which align well with the values reported in the literature. These findings establish a direct link between large-scale flow structures and potential environmental heterogeneities, such as an oxygen deprivation zone, providing a macroscopic perspective that supports process understanding and the development of scale-down model design.

RQ3.3: Critical flow structures such as up- and down-pumping zones, a poorly mixed region in the top part of the STR, circulation times, residence times, and mixing time scaling laws are captured consistently using experimental LSPs. The combined experimental and numerical analyses confirm a consistent compartmentalization into three distinct regimes. A regime-based lifeline analysis allows estimating the probability of stress-related conditions, such as oxygen deprivation. These findings not only demonstrate the technological readiness of LSPs for industrial bioreactor characterization and their suitability for computational fluid dynamics (CFD) validation, but also highlight their potential as a leading experimental tool to supply industrial-scale fluid dynamic data for scale-down model development.

Future directions

Despite promising results, several challenges remain in the application and further development of LSPs and the corresponding simulation techniques.

Current LSPs are relatively large and do not behave as flow tracers, particularly in low-viscosity media or in smaller bioreactors, such as single-use bioreactors with volumes up to 2000 L. Advancements in passive sensor technologies, such as magneto-mechanical resonators, could enable the development of smaller particles. The tracking of particle position and orientation in all three spatial dimensions has been demonstrated, although reliable oper-

ation over longer distances remains to be demonstrated [Mer25]. Additionally, employing radio-frequency or optical energy harvesting approaches could eliminate the need for batteries and create space for more onboard sensors. Regarding complete three-dimensional (3D) reconstruction, conventional positioning technologies such as Bluetooth, WiFi, ultra-wide band, or ultrasound are limited in steel reactor environments due to signal attenuation in the media, signal interference, signal shadowing from obstacles, and the requirement for line-of-sight conditions [Lau19a, Tod13a]. In contrast, Inertial Measurement Units (IMU) allow autonomous tracking but suffer from integration drift over time, making trajectory reconstruction challenging and highlighting the unresolved problem of accumulated position errors over time [Bun23]. Recent developments have led to a Python-based framework capable of processing both analytically defined and experimentally measured velocity fields to reconstruct continuous flow fields from Particle Image Velocimetry (PIV) data, enabling the simulation of particle trajectories using the Maxey–Riley equation [Uri23]. Despite these challenges, embedding sensors in LSPs and using them to map hydrodynamic compartments equips bioprocess engineers with a powerful tool to diagnose bioreactor performance in detail. Integrating lifeline-based data into early stage process development promotes more predictive, robust, and sustainable bioprocesses. Thereby, the single multicompartment bioreactor (SMCB) platform holds the potential to enable controlled laboratory-scale design and investigation of large-scale process conditions. Future research should aim to enhance the current compartment model by incorporating additional large-scale flow information and exploring the effects of varying power inputs [Gau24].

On the simulation side, further progress is required in data synthesis. A key challenge in comparing simulations with experimental data is the ergodicity problem: current experimental LSP trajectories are typically long, while simulations generate numerous short trajectories, complicating direct statistical comparisons [Fei25]. Solving this issue will require access to more graphics processing unit (GPU) resources to reduce the current wall clock time of five to seven days per simulation (for single-phase mixing). Moreover, introducing additional model assumptions may worsen the agreement with experiments. For instance, neglecting the time history (Basset) force already leads to significant differences in particle clustering patterns in flows with moderate to large Stokes numbers [Uri25]. Additionally, for simulations of inertial particles, appropriate grid resolutions and sub-grid scale models must be selected to capture particle dynamics, especially on industrial scales. Future work must define best practices while ensuring computational feasibility. Although this study provides fundamental information on hydrodynamic heterogeneities in single-phase bioreactors, extending these findings to gas–liquid systems remains a major challenge due to added complexities such as bubble size distributions, buoyancy-driven heterogeneities, and the validation of mixing

Conclusion and outlook

times. Incorporating gas–liquid interactions into LB LES remains computationally intensive and is an ongoing area of research. A recently introduced trajectory-based breakup model offers improved treatment of these interactions [Wei24]. Future models necessitate to link mass transfer coefficients with bubble deformation and need further validation. Ultimately, integrating these advances to accurately represent real fermentation conditions is a logical next step.

Overall, this thesis demonstrates that LSPs provide a powerful experimental approach to map hydrodynamic compartments and lifelines in bioreactors, offering insights that are difficult to obtain with conventional sensors. By enabling detailed monitoring of substrate gradients, and the effects of operational changes such as impeller frequency or aeration, LSPs support the optimization of process performance and robustness. Combined with validated simulations, they open a new perspective on the design of future bioreactors, including highly instrumented or self-regulating systems, and pave the way toward more predictive and efficient industrial bioprocesses [CRC25].

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Appendix A

Repositories for raw files, calculations, and result files according to FAIR principles

Documentation and public access to research results

Good scientific practice requires that research data, methods, and codes are documented transparently, reproducibly, and without selection or manipulation. Wherever possible, results and underlying data are made publicly accessible in recognized repositories, following the FAIR principles (findable, accessible, interoperable, reusable). This not only supports verification and reuse by others but also strengthens transparency and sustainability of research. In interdisciplinary fields such as biotechnology, structured and collaborative research data management plays a central role in keeping data available throughout the entire data lifecycle [Deu22, Reh23].

This appendix provides an overview of the saved repositories for raw data, calculations, and result files in accordance with the FAIR principles, including a brief outline of the structure and contents of the respective repositories as well as the corresponding links.

Data for: Lagrangian sensors in a stirred tank reactor: Comparing trajectories of 4D-Particle Tracking Velocimetry and Lattice-Boltzmann simulations

- Gitlab Repository: <https://collaborating.tuhh.de/v5/multiphase-bioreactors/sebastian-hofmann/cej-data-4dptv-lbsimu>
- Description: This dataset comprises exemplary segments of raw data and the complete MATLAB code covering the following topics: (1) raw data handling of output files from DaVis (4D-PTV) and M-Star (Lattice-Boltzmann large eddy simulation) for particle tracking in a stirred tank reactor, (2) Lagrangian evaluations of velocity and acceleration, (3) Eulerian evaluations of Reynolds particle numbers and Stokes numbers, and (4) additional calculations and evaluations based on numerically determined data to complement the experimental results.
- Relevant folders:
 - /data
 - * ./code: Contains all MATLAB scripts for experimental and simulated raw data handling, statistical evaluations of velocity and acceleration data, force distribution calculations, particle Reynolds number estimation, velocity vector binning, Stokes number estimation, particle distance calculations, and related analyses.
 - * ./sample_raw_data: Provides sample raw data (*.txt files) from experiments and simulations, as well as an Excel file with Shake-The-Box particle statistics results.
 - /figures: Contains all figures included in the publication.
- DaRUS repository: <https://doi.org/10.18419/darus-2294>

Data for: Experimental analysis of lifelines in a 15,000 L bioreactor by means of Lagrangian Sensor Particles

- Gitlab Repository: <https://collaborating.tuhh.de/v5/multiphase-bioreactors/sebastian-hofmann/cherd-data-for-lsp-lifeline-analysis>
- Description: This dataset comprises raw data (including an overview of selected LSP datasets and metadata), MATLAB code, and results. It provides data processing and analysis of Lagrangian Sensor Particle (LSP) data in a 15,000 L bioreactor and covers the following topics: (1) initialization of conditions, (2) computation of various physical properties, and (3) iteration over datasets corresponding to experimental data. It further includes data selection, logging, visualization, and storage functionalities.
- Relevant folders:
 - /code: Contains all MATLAB scripts for experimental data handling. The `main.m` file integrates all functions denoted `f_functionname.m`. Calculations include preprocessing and filtering, axial velocity computation, probability density functions (PDFs) over reactor height, binned axial velocity computation, volumetric flow rate and residence time analysis in bins, PDF calculation for axial velocity, circulation time determination, compartment identification, and Lagrangian regime analysis. A separate script handles the plotting of all results.
 - /raw_data/HZDR: Contains all raw files (*.npz and *.mat) from the DaRUS repository, the associated metadata file for handling experimental LSP data, and a README with an overview of all utilized experiments.
 - /results/results_HZDR:
 - * ./data: Contains *.mat result files and a log file of evaluated datasets.
 - * ./figs: Contains the corresponding result figures.
- DaRUS repository: <https://doi.org/10.18419/darus-3768>

Data for: Lagrangian Sensor Particles for detecting hydrodynamic heterogeneities in industrial bioreactors - experimental analysis and Lattice-Boltzmann simulations

- Gitlab Repository: <https://collaborating.tuhh.de/v5/multiphase-bioreactors/sebastian-hofmann/CEJA-data-for-comparison-of-lsp-trajectories-with-lb-simulation>
- Description: This dataset comprises raw data (with an overview of selected LSP datasets and metadata), MATLAB code, and results. It provides data processing and analysis of Lagrangian Sensor Particle (LSP) data together with accompanying numerical simulations in a 15,000 L bioreactor. The following topics are implemented: (1) alignment of experimental and numerical data via “adapters”, (2) initialization of conditions, (3) computation of various physical properties, and (4) iteration over datasets combining experimental and numerical data. In addition, it includes data selection, logging, visualization, and storage functionalities.
- Relevant folders:
 - /code: Contains all MATLAB scripts for data handling. The `main.m` file integrates all functions denoted `f_functionname.m`. Calculations include condition and parameter setup, preprocessing and calculation of experimental data, handling of Lattice-Boltzmann large eddy simulation (LB LES) data, preparation of experimental LSPs, tracers, and simulated LSPs, as well as a data analysis loop (with functions such as axial velocity calculation, probability density functions (PDFs) over reactor height, residence time calculation in bins, and others). It further enables reloading of *.mat data, saving indexed datasets for plotting, and conducting a sensitivity study on numerical data including particle statistics for LB LES at different volume fractions. Final calculations include Stokes number grids, particle Reynolds number grids, and theoretical particle sizes. Additionally, `main_mix_time.m` handles global and local mixing time evaluation (using `f_create-validate_mask.m`, `f_loc_mix_time.m`, and `f_glob_mix_time.m`), loads and saves masks videos, and calculates mixing times iteratively. A separate script handles all plotting tasks.
 - /raw_data: Contains all raw files and Excel files providing overviews of LSP recordings and mixing time trials, including information on accompanying conductivity trials (not part of this publication and thesis). Main raw files are available in the DaRUS repository.

-
- * `./HZDR`: Contains all *.mat raw files, the associated metadata file, and an overview of LSP data used for evaluation.
 - * `./LBS`: Contains a README with a file table, a metadata file for handling all raw simulation files, and multiple folders with *.mat simulation data. Sensitivity study datasets are labeled `sens_study_xxx`.
 - * `./WTW`: Contains raw files of conductivity measurements (not used).
 - * `./mixing`: Contains a README describing video handling and cutting, a metadata file, a bash script that loops through all entries in `cuts.txt` to trim videos, and all raw video files of mixing time trials.
 - `/results`: Contains all intermediate and final result files.
 - * `./results_HZDR`: Contains individual data results and figures, including raw evaluations of each dataset, and a log file of evaluated datasets.
 - * `./results_HZDR_LBS`: Contains all combined results from experiments and simulations, as well as final figures.
 - * `./results_LBS`: Contains individual simulation results and figures, including raw evaluations of each simulated dataset, and a log file of evaluated datasets.
 - * `./results_mixing`: Contains results of global and local mixing time evaluations, including the binary mask used.
 - DaRUS repository: <https://doi.org/10.18419/DARUS-4529>

