AN INTEGRATED NUMERICAL AND EXPERIMENTAL INVESTIGATION OF THE FLOW AND POWER CONSUMPTION IN SCALE-DOWN BIOREACTORS

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I, Artemis Danae Charalambidou, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature
To my parents and Antonis
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Abstract

Optimal mixing performance is of major importance for the chemical and biochemical industries. The design of economically feasible and profitable production processes has driven companies towards optimisation of continuous manufacturing and intensification of individual operations by reducing working volumes and increasing operating frequencies. In this respect, the development of robust small scale devices capable of multivariate process optimisation is essential.

The aim of this work is to characterise the flow pattern and power consumption in a 250 ml-scale stirred tank reactor (STR) operating at \( Re = 3,732 \). Velocity and turbulence characteristics are computed via Computational Fluid Dynamics (CFD) and validated via Particle Image Velocimetry (PIV) for radially agitated unbaffled (UB) and baffled configurations equipped with baffles of different sizes. Trailing vortex stability is assessed with respect to baffle presence and size while the impact of the impeller disk on flow instabilities is discussed. Proper Orthogonal Decomposition (POD) and Fast Fourier Transform (FFT) are used to extract dominant spatial modes and corresponding frequencies affecting the underlying flow patterns.

Results showed good agreement between CFD and PIV while POD analysis revealed the existence of highly energetic and periodic modes, linked to interactions between impeller jet and reactor walls. These modes are responsible for an impeller jet instability, which is amplified by baffle presence, size and absence of impeller disk. Assessment of power consumption across various small scale bioreactors showed that the presence of probes results in a power number rise equivalent to that produced by baffles, more pronounced in radially agitated vessels. Flow characterisation around the probes highlights that changes in probe geometry can lead to power number reduction.

This study demonstrates that thorough understanding of the hydrodynamics is important for parameter optimisation when small scale reactors are tested as minor design changes can affect the flow characteristics, thus having significant implications on mixing and mass transfer performance.
Impact Statement

The biopharmaceutical industry is facing constant pressure to decrease manufacturing costs and increase process knowledge to effectively intensify production processes, improve product quality and lower development timelines. The work presented in this thesis aims to address the aforementioned challenges in the context of engineering optimisation of scale-down models (SDMs), operating in continuous mode, and provide an integrated framework based on a set of computational and experimental tools to assist in the evaluation of bioreactor engineering features underpinning mixing processes and the generation of more effective scaling protocols.

Small scale bioreactors are considered appropriate candidates for the optimisation of bioprocesses, therefore thorough understanding of hydrodynamics in their interior will contribute in optimising vessel geometry without compromising mixing performance. This work provides an end-to-end characterisation of hydrodynamics in a SDM successfully used in perfusion CHO cell cultures. To this end, an experimentally validated CFD methodology is described which was used to effectively study the flow patterns developed at intermediate Reynolds numbers, in which bioreactors at mL-scale commonly operate, and examine the way critical geometrical features affect the resulting flow field. From an academic perspective, the development of a process implementing validated CFD tools provides a baseline for the procedural knowledge needed to assess novel impeller designs and bioreactor configurations at small scales, but also forms an example for the examination of geometrically similar larger scale systems. The use and dissemination of such methodologies can be applied as precursors for analogous studies by other researchers and contribute to increasing the quality of the acquired data in reasonable timescales.

Scaling remains a challenging aspect of bioprocessing, often associated with considerable expenditure of time and resources. Power per unit volume is a commonly used scaling criterion but it is usually derived from published empirical data, often obtained for vessels operating at pilot or larger scales. Results in this work prove that this approach is often not appropriate in SDMs as available data corresponding to standardised reactor geometries lead to erroneous values for power consumption since especially at smaller scales the presence of internals, including probes and/or baffles, might significantly impact the hydrodynamic environment.
Outside academia, the findings outlined in this work can serve as a proof of concept for the way bioreactor geometry and internals affect power characteristics, assisting in the development of robust scale-down designs, able to operate equivalently to larger scales. Combined experimental and CFD results can provide the industry with useful information with respect to the strengths and weaknesses associated with the different models and their ability to effectively reproduce the generated flow. Moreover, flow characteristics in the presence of internals can shed light on the design of optimised geometries for the latter, which is promising for the reduction of energy demands, an aspect having great implications on high cell densities cultures.
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# Nomenclature

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<td>2D</td>
<td>Two dimensional</td>
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<tr>
<td>3BS</td>
<td>3-Blade segment</td>
</tr>
<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>ATF</td>
<td>Alternating tangential flow</td>
</tr>
<tr>
<td>BPF</td>
<td>Blade passage frequency</td>
</tr>
<tr>
<td>CCW</td>
<td>Counter-clock wise</td>
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<tr>
<td>CFD</td>
<td>Computational fluid dynamics</td>
</tr>
<tr>
<td>CHO</td>
<td>Chinese hamster ovary</td>
</tr>
<tr>
<td>COGs</td>
<td>Cost of goods</td>
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<td>CPPs</td>
<td>Critical process parameters</td>
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<td>CQAs</td>
<td>Critical quality attributes</td>
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<td>DES</td>
<td>Detached eddy simulations</td>
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<tr>
<td>EA</td>
<td>Ensemble average</td>
</tr>
<tr>
<td>FBT</td>
<td>Flat Blade Turbine</td>
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<tr>
<td>FDA</td>
<td>Food &amp; Drug administration</td>
</tr>
<tr>
<td>FFT</td>
<td>Fast Fourier Transform</td>
</tr>
<tr>
<td>GMP</td>
<td>Good manufacturing practices</td>
</tr>
<tr>
<td>HEK</td>
<td>Human embryonic kidney</td>
</tr>
<tr>
<td>HT</td>
<td>High-Throughput</td>
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<tr>
<td>ICB</td>
<td>Integrated continuous biomanufacturing</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>LDA</td>
<td>Laser Doppler anemometry</td>
</tr>
<tr>
<td>LES</td>
<td>Large eddy simulations</td>
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<tr>
<td>LOM</td>
<td>Low order model</td>
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<tr>
<td>mAb</td>
<td>Monoclonal antibody</td>
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<tr>
<td>MIs</td>
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<td>MRF</td>
<td>Multiple reference frame</td>
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<td>NI</td>
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<tr>
<td>NPV</td>
<td>Net present value</td>
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<tr>
<td>P₁</td>
<td>Vertical plane between baffles</td>
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<td>P₂</td>
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<td>PBT</td>
<td>Pitched blade turbine</td>
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<tr>
<td>PIV</td>
<td>Particle image velocimetry</td>
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<td>POD</td>
<td>Proper orthogonal decomposition</td>
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<tr>
<td>QbD</td>
<td>Quality by design</td>
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<tr>
<td>Q&lt;sub&gt;cr&lt;/sub&gt;</td>
<td>Q-criterion</td>
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<tr>
<td>rms</td>
<td>Root mean square</td>
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<td>RSM</td>
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<td>RT</td>
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<td>SAS</td>
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<td>SDM</td>
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<td>SM</td>
<td>Sliding mesh</td>
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<tr>
<td>STR</td>
<td>Stirred tank reactor</td>
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<tr>
<td>UB</td>
<td>unbaffled</td>
</tr>
<tr>
<td>(U)RANS</td>
<td>(Unsteady) Reynolds averaged Navier-Stokes equations</td>
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</table>
Roman symbols

\( A \quad (m^2) \) \quad Area

\( B \quad (m) \) \quad Baffle width

\( B_1 \) \quad Small baffle width \((D_T/16)\)

\( B_2 \) \quad Large Baffle width \((D_T/10)\)

\( C \quad (m) \) \quad Impeller clearance

\( C_D \) \quad Drag coefficient

\( C_S \) \quad Smagorinski constant (LES)

\( D_i \quad (m) \) \quad Impeller diameter

\( D_T \quad (m) \) \quad Tank diameter

\( d_p \quad (m) \) \quad Seeding particle diameter

\( f \quad (Hz) \) \quad Frequency

\( F \quad (N) \) \quad Force

\( F_D \quad (N) \) \quad Drag force

\( G_k \quad (m^2/s^3) \) \quad Production of \( k' \) due to velocity gradients \((k-\varepsilon \text{ models})\)

\( G_b \quad (m^2/s^3) \) \quad Production of \( k' \) due to buoyancy \((k-\varepsilon \text{ models})\)

\( H_L \quad (m) \) \quad Liquid height

\( k \quad (m^2/s^2) \) \quad Kinetic energy

\( k' \quad (m^2/s^2) \) \quad Turbulent kinetic energy

\( k_L a \quad (h^3) \) \quad Oxygen transfer coefficient

\( k_{per} \quad (m^2/s^2) \) \quad Periodic kinetic energy

\( l_o \quad (m) \) \quad Integral macroscale

\( l \quad (m) \) \quad Chord length

\( l_A, l_B, l_C \quad (m) \) \quad Probe length
$l_r$ (m)  Lever arm

$M$ (Nm)  Moment

$N$ (rpm)  Impeller rotational speed

$N_s$  Number of samples (PIV frames)

$n$  Number of angles $\varphi$ for which phase resolved data was obtained

$n_b$  Number of baffles

$n_{\varphi}$  Number of samples corresponding to a certain phase angle $\varphi$

$P_1$  Vertical plane between baffles

$P_2$  Vertical baffle plane

$P$ (W)  Power consumption

$P_a$  Small probes

$P_b$  Big probes

$p$ (Pa)  Pressure

$P_0$  Power number

$R$ (m$^2$/s$^2$)  Reynolds stress

$R_{Pa}$ (m)  Probe radius

$Re$  Reynolds number

$S_{ij}$ (1/s)  Rate of strain tensor

$Sr$  Strouhal number

$t$ (s)  Time

$t_{baffle}$ (m)  Baffle thickness

$t_{blade}$ (m)  Impeller blade thickness

$|u|$ (m/s)  Velocity magnitude

$u_i$ (m/s)  Average velocity component
\( u_i(\varphi,t) \) (m/s) Instantaneous velocity component
\( u'_i \) (m/s) Fluctuating velocity component
\( \langle u_i \rangle \) (m/s) Phase resolved velocity component
\( u_r \) (m/s) Radial velocity component
\( u_\theta \) (m/s) Tangential velocity component
\( u_{\text{tip}} \) (m/s) Impeller tip velocity
\( u_z \) (m/s) Axial velocity component
\( U_\infty \) (m/s) Free stream velocity
\( V \) (m\(^3\)) Volume
\( V(\%) \) Probe blockage ratio
\( w_b \) (m) Impeller blade width

**Greek symbols**

\( \alpha \) (°) Angle around the probe circumference
\( \alpha_\varepsilon \) Inverse effective Prandtl number for \( \varepsilon \) (k-\( \varepsilon \) RNG)
\( \alpha_k \) Inverse effective Prandtl number for \( k' \) (k-\( \varepsilon \) RNG)
\( a_n \) (m/s) \( n^{th} \) POD temporal eigenfunction
\( \beta \) (°) Angle of the impeller jet
\( \delta \) (m) Thickness of the streamlined probe
\( \Delta \) (m) Grid spacing
\( \varepsilon \) (m\(^2\)/s\(^3\)) Energy dissipation rate
\( \eta \) (m) Kolmogorov length scale
\( \lambda \) (m) Taylor microscale
\( \lambda_i \) (m\(^2\)/s) Energy of mode i
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<th>Symbol</th>
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<tr>
<td>$\mu$</td>
<td>(kg/ms)</td>
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$\Omega_{ij} \quad (1/s) \quad \text{Rate of rotation tensor}$
Chapter 1: Introduction

1.1 The context of the research

Monoclonal antibodies (mAbs) have been developed commercially since 1980. The first licensed mAb was muromonab-CD3 (trade name Orthoclone OKT3, launched by Janssen-Cilag) and was approved in 1986 in the United States for the prevention of kidney-transplant rejection. To date, major progress in engineering, genetic sequencing and translation of medical sciences into clinical practice have rendered mAbs the most rapidly growing biotechnology-derived therapeutic molecules. More than 25% of the total recombinant-protein products launched in 2019 were mAbs for the treatment of multiple diseases from viral infections to cancers (Liu, 2014; Ecker, Crawford and Seymour, 2020). In 2021 the global mAbs market was valued at US$177.46 billion and it is expected to increase to US$524.68 by 2030 (Precedence Research, 2022). Almost 70% of mAbs produced by mammalian cells use Chinese Hamster Ovary (CHO) cells as expression platform, due to their adaptability in different culture conditions and serum-free media eliminating the contamination and variability among different batches (Orellana et al., 2015; Dumont et al., 2016).

Besides the rapid economic growth and profitability of the mAbs market, monoclonal antibody treatments are associated with high costs compared to other therapeutics. Typical prices per patient for specific treatments range from US$5,000-US$8,000 with some products (such as Herceptin) reaching US$30,000. The increasing pressure from healthcare providers and the anticipated capacity shortage have led to the reduction of manufacturing costs at the production scale and encouraged the investigation of alternative manufacturing technologies (Farid, 2006). The design of economically feasible production processes has driven leading biotech companies to move towards continuous processing, which benefits from a reduction of working volumes, high frequency operations and smaller batches (Croughan, Konstantinov and Cooney, 2015). Continuous mode in upstream processing is associated with a constant addition of nutrients and product removal with cells being retained in the culture by a cell retention device, a process which is named perfusion. Perfusion is estimated to increase production capacity by operating at high cell densities for elongated time scales which is expected to decrease the reactor working volumes by 1 to 2 orders of magnitude and consequently reduce initial capital costs (Croughan, Konstantinov and Cooney, 2015). Although to date more than 17 commercially available biopharmaceuticals have been
produced by perfusion mode, studies have shown that it is related to a high risk of failure, throwing doubts on the reliability of the method and underlining the necessity of thorough studies and optimisation (Coffman et al., 2021).

Clinical manufacturing and process development have been estimated to comprise approximately 17% of the total R&D costs. Therefore, it is essential to evaluate the relationship between cost reduction and the process development adaptation necessary in order to achieve it. This is particularly important when novel technologies and operations are considered (Mahal, Branton and Farid, 2021). In this context, Scale-Down Models (SDMs) and High-Throughput (HT) technologies are increasingly employed within the biopharmaceutical industry. Small scale mimics of manufacturing scale processes are associated with the reduction of cost and time consuming large scale engineering studies and are essential tools for the in-depth characterisation of process operating parameters by enabling small volume operations and allowing the use of multiple systems in parallel. These systems have sufficient automation for adequate feed exchange and continuous aeration allowing the operation at high cell density cultures and increasing significantly the capacity of scale-down systems far beyond manual devices (Bareither and Pollard, 2011).

To successfully replicate upstream bioprocesses in small scale systems, it is important that comparability of Critical Quality Attributes (CQAs) and the controlling Critical Process Parameters (CPPs) across scales is demonstrated (Sandner et al., 2019). Investigation of engineering characteristics in small scale vessels can be beneficial for the identification of design parameters affecting the flow patterns and ultimately mixing and suspension mechanisms. In this respect, the development of validated CFD tools could further improve the efficiency of characterisation studies, giving access to 3D data throughout the entire bioreactor volume (Chalmers, 2015). While validation of CFD modelling in reactor systems has been discussed in the literature (Hartmann et al., 2004; Delafosse et al., 2008; Gimbun et al., 2012; Collignon et al., 2016), the detailed characterisation of hydrodynamics is limited to pilot or larger scales with fixed design ratios. On the other hand the design characteristics in reactors of smaller volumes, including sizing parameters, tank and internal geometry, are rarely geometrically similar to larger scale systems which is a source of discrepancies in the physical environment between small and large volumes, affecting the culture outcomes and their comparison across scales (Nienow, Scott, et al., 2013; Collignon et al., 2016; Rotondi et al., 2021; Charalambidou, Micheletti and Ducci, 2022).
The motivation of the present work stems from the importance of the systematic understanding of the hydrodynamics in small scale bioreactors in order to improve scaling and optimise the design of qualified SDMs. To this end, the investigation of the ways bioreactor features influence the average and instantaneous flow quantities impacting the overall velocity distribution, turbulence levels, flow stability and eventually scaling parameters, is essential. Scientific insights and methodologies previously employed for the understanding and optimisation of mixing processes in stirred vessels with standard geometries (Escudié, Bouyer and Liné, 2004; Delafosse et al., 2008; Ducci, Doulgerakis and Yianneskis, 2008; Bouremel, Yianneskis and Ducci, 2009a; Doulgerakis, Yianneskis and Ducci, 2011) are leveraged and expanded to smaller scales, aiming to contribute to the formulation of a framework involving an end-to-end characterisation of engineering aspects across different STR volumes. The acquired engineering and hydrodynamics knowledge provides a baseline to assess how critical design parameters might affect the fluid dynamics in small scale reactors and can then be used to assist in the optimisation of mixing, mass transfer and scale-up.

In the following section, a detailed literature review concerning the aforementioned studies on the engineering aspects of small and large scale STRs are discussed and used to formulate the aim of this project. An outline of the current thesis is presented at the end of this chapter in Section 1.5.

1.2 Literature survey

In this section a literature survey is conducted about the history and modes of the upstream cell culture operation and the subsequent development of scale-down methodologies for continuous biomanufacturing focusing on perfusion cultures for mammalian cells. The existing published contributions focusing on the engineering characterisation of small scale bioreactors are reviewed and summarised. Experimental and computational methodologies for the systematic evaluation of the flow field in stirred tanks are described, with attention to the impact of reactor geometrical features on the resulting flow patterns determining mixing and suspension mechanisms. Finally, limitations in STR scale-up/down are discussed focusing on the impact of bioreactor internals, i.e. baffles and/or probes, on power consumption.
1.2.1 Production of biopharmaceuticals from mammalian cells

Protein therapeutics including monoclonal antibodies (mAbs), recombinant proteins and peptides, characterise the largest group of new products developed by the biopharmaceutical industry (Dumont et al., 2016). Mammalian cells have been a fundamental source for the production of protein therapeutics and mAbs due to their posttranscriptional metabolic pathways, which can support the production of human compatible complex biomolecules, and highly glycosylated proteins (Zhang, 2010). Several mammalian cell lines have been used in bioprocessing including human embryonic kidney (HEK293), mouse myeloma (NS0) cells and Chinese hamster ovary (CHO) cells, with the latter being the predominant cell line due to ease of handling and robustness across different conditions and scales (Moo-Young et al., 2011; Hacker and Balasubramanian, 2016). Manufacturing of biopharmaceuticals typically occurs in suspension systems due to advantages of homogeneity, simplicity and ease of implementation of production protocols in different scales (Birch and Arathoon, 1990).

Stirred tank reactors (STRs) are mostly used for the production of recombinant proteins from mammalian cell lines across a wide range of scales (ranging from mL to thousands of litres) (Andersen and Reilly, 2004; Khopkar and Ranade, 2006; Zhang, 2010). The selection of scale during the manufacturing process depends on the targeted production yield and mode of operation. Fed-batch processes are most commonly used for the production of mAbs due to their robustness, reproducibility, scalability and reliability of manufacturing outcomes. In fed-batch culture nutrients are added continuously or as bolus at certain time points during the culture (Wiegmann et al., 2019). Continuous bioprocess optimisation, media improvement and development of high throughput small scale systems have led to a twenty-fold increase in volumetric and specific productivities of fed-batch cultures with titers routinely exceeding ~8-10 g/L (Wuest, Harcum and Lee, 2012; Lin et al., 2019; Xu et al., 2020). Even though the capability of the latter to generate high titres has been demonstrated, the process throughput is restricted by the limited capacity to increase bioreactor units while the product quality may be compromised by the long residence times. Continuous manufacturing has progressively attracted the scientific and industrial interest as an alternative manufacturing route to address the aforementioned challenges and increase bioprocess throughput while reducing capital costs and increasing process flexibility (Croughan, Konstantinov and Cooney, 2015).

1.2.2 Continuous biomanufacturing

Biopharmaceutical companies often need to amenably accommodate the production of large, medium and small volume drugs and therapeutic proteins, ideally within the same
manufacturing facility. Moreover, the growing competition from biosimilar products, in combination with the rapid adjustment of production capacities to support emerging markets, have raised concerns about the lasting sustainability of conventional batch manufacturing prototypes consisting of multiple batch bioreactors and downstream trains (Konstantinov and Cooney, 2015). Following the major advances in cell engineering, there is increasing interest towards the implementation of intensified processes based on continuous media addition and simultaneous removal of toxic by-products inhibiting cell cultures, characterised by equal or higher achievable productivities operating with smaller volumes (Yang et al., 2016; Janoschek et al., 2019).

Continuous mode in cell culture has been used for many years, mainly for the manufacturing of labile proteins including coagulation factors and enzymes in which continuous harvest is required to ensure optimal product quality (Kelley, Jankowski and Booth, 2010; Girard et al., 2015; Arnold et al., 2019). The simultaneous evolution of perfusion processes in upstream and optimisation of continuous protein purification in downstream processing have assisted in the development and increasing application for integrated continuous biomanufacturing (ICB) for the production of biopharmaceuticals from mammalian cell lines (Arnold et al., 2019; Coffman et al., 2021). The renewed interest towards ICB can be attributed to its potential to intensify production yields and equipment throughput while simultaneously leading to improved product quality compared to conventional batch processes (Coffman et al., 2021; Mahal, Branton and Farid, 2021). Cost advantages for ICB over batch operation have been examined in the past for both commercial and clinical manufacturing. Even though continuous bioprocessing is linked to increased volumetric productivity, the overall economic analysis on the cost of goods (COG) has given variable outcomes depending on numerous parameters including the number and volume of equipment units, facility design, process duration, media use but also manufacturing flexibility and automation (Pollock, Ho and Farid, 2013; Konstantinov and Cooney, 2015; Arnold et al., 2019). Moreover, process intensification through ICB has been supported by the FDA as a robust way to tackle severe existing diseases and potential future outbreaks, due to the flexibility of implementation and cost efficiency (Gjoka, Gantier and Schofield, 2017).

1.2.2.1 Continuous manufacturing in upstream processing: Perfusion culture

In upstream bioprocessing, strategies for perfusion culture implementation are linked to increased daily productivity and reduced facility footprints compared to batch and fed-batch processing. Nevertheless, the expansion of their application has been hindered due to issues
related to validation and operation complexity in addition to high likelihood of technical failure (Pollock, Ho and Farid, 2013). Recent technology advances have contributed to minimising process failures and enabling intensified production processes with prolonged culture operations at constant viable cell densities which make perfusion a promising alternative for mammalian cell cultivation (Hiller et al., 2017; Wolf et al., 2019; Tregidgo, 2021).

Due to continuous replenishment of fresh nutrients, perfusion cultures are characterised by cell densities which are 1 to 2 orders of magnitude higher compared to fed-batch cultures ($10^7$-$10^8$ cells/mL versus $10^6$-$5 \times 10^6$ cells/mL) while a ten-fold increase in volumetric productivities has been demonstrated (Bibila and Robinson, 1995; Lim et al., 2006). Typically, it is reported that constant cell density perfusion cultures have been associated with productivities of 2 to 4 g/L per day at $7 \times 10^7$ cells/mL with a duration of ~70 days or more (Brower, Hou and Pollard, 2015; Coffman et al., 2021). Even though perfusion mode is linked to high productivities, it is also associated with reduction in reactor capacity required (10,000-20,000 in fed-batch versus 500-1000 L in perfusion), thus allowing lower initial investments (Lim et al., 2006; Farid, Thompson and Davidson, 2014; Croughan, Konstantinov and Cooney, 2015; Mahal, Branton and Farid, 2021). Another advantage of smaller volume perfusion systems is the potential of utilisation of single-use technologies (SUT), which further broadens the process flexibility allowing for the development of facilities that can accommodate the manufacturing of multiple products (Mahal, Branton and Farid, 2021). The aforementioned advantages, alongside the increased product quality and the relevant instrumentation and automation associated with perfusion mode, can justify the increasing interest towards the optimisation and implementation of perfusion processes for the production of biopharmaceuticals and monoclonal antibodies.

Several studies have focused on the evaluation of the economics of perfusion operations by comparing it with fed-batch culture (Pollock et al., 2017; Farid and Mahal, 2019; Coffman et al., 2021; Mahal, Branton and Farid, 2021). Despite its advantages in productivity and capital investments, the resources needed for implementation of perfusion often lead to an increase of the operating costs imposing concerns with respect to the overall economic feasibility of the process. Lim et al. (2006) performed a computer aided approach based on a case study for the determination of process economics between fed-batch and perfusion modes for mAbs production and reported a 42% reduction in capital investment alongside a 12% increase in net present value (NPV) for the latter. In the same work, while the economic feasibility was proven to be higher for perfusion, the potential of high failure rates made the process less
robust than traditional fed-batch culture (Lim et al., 2006). Similarly, Pollock et al. (2013) compared the economics between perfusion and fed-batch processes and suggested that the robustness of perfusion processes is dependent on its methodology (i.e. ATF versus spin filter with the former being more robust) but underlined the advantage of fed-batch on ease of operation and validation (Pollock, Ho and Farid, 2013).

1.2.3 Small scale Bioprocessing

The need to reduce cost of biopharmaceuticals per patient has driven biotechnology companies to optimise process efficiency and decrease process development time scales. This challenge is accompanied by the need for acceleration of upstream experimentation to comply with quality by design (QbD) principles and development of predictive models of systems biology (Bareither and Pollard, 2011). The development and qualification of scale-down models (SDMs) are important for the understanding of critical processes to support process validation and commercial manufacturing (Tozer et al., 2005). Moreover, SDMs assist in the optimisation of high throughput operations, allowing the conduction of several experiments in parallel to explore the multivariate design space and contribute to the reduction of cost and time associated with parameter optimisation and process development.

An assortment of small scale devices are commercially available for scale-down studies for upstream bioprocessing accompanied by either low or high levels of instrumentation such as microwell plates and small scale bioreactors respectively. The working volume of scale-down devices commonly corresponds to mL-scale, but there are commercially available options with volumes up to several litres (<10 L) (Doig, Baganz and Lye, 2006). Several pharmaceutical companies developed in the past their own scale-down devices, aimed at either mimicking standalone operations or the entire bioprocess sequence, in order to facilitate process optimisation and understanding of the multiple interacting variables underpinning the individual unit operations (Doig, Baganz and Lye, 2006; Micheletti and Lye, 2006; Sandner et al., 2019).

Scale-down devices, used in upstream microscale bioprocessing, include shake flasks, microwell plates, microreactors from the application of microfluidics and small scale bioreactors (Betts and Baganz, 2006; Micheletti and Lye, 2006). Shaken systems allow the parallelisation of experiments and the simultaneous investigation of multiple variables, thus have been extensively used in primary screening during process development applications (Micheletti and Lye, 2006). Considering that the industry standard for large scale
fermentations is the stirred tank (bio)reactor, discrepancies in process performance are often observed when transitioning from small to larger scales. These changes in cell culture performance are attributed to the physical differences between stirred tanks and shaken systems including the mixing (i.e. shaken versus mechanically stirred) and gassing (i.e. surface aeration versus gas sparging) strategies in combination with the lack of online monitoring and control often absent in well systems and flasks (Nienow, Rielly, et al., 2013a). Although previous works presented the application of shake flasks equipped with the relevant instrumentation for online monitoring of dissolved oxygen and pH (Anderlei and Büchs, 2001; Wittmann et al., 2003), their operation is complicated and their use in industry is limited (Nienow, Rielly, et al., 2013b). The use of small scale (mL-scale) bioreactors can overcome the aforementioned limitations both in terms of preserving the physical aspects between scales (i.e. agitation and sparging) and allowing the continuous monitoring and control of key parameters affecting culture performance.

To date several bioreactors options are (commercially) available for small scale batch culture bioprocessing (Kim, Diao and Shuler, 2012; Nienow, Scott, et al., 2013; Rotondi et al., 2021) and fed-batch processes (Kim, Diao and Shuler, 2012; Velez-Suberbie et al., 2018), however options are still limited for perfusion operation due to sophisticated instrumentation needed and advanced operational requirements; though increasing number of SDMs are in development for perfusion cultures. Considering that the establishment of an optimised perfusion culture is characterised by long time scales, high costs and increased complexity due to operation in high cell densities, SDMs are an attractive tool which can be used to effectively address potential bottlenecks in perfusion culture and improve its performance in a cost-and time-effective way.

1.2.3.1 Small scale bioreactors as Scale-Down Models
Small scale bioreactors have been initially used in early stage clinical development as screening tools, but there is growing interest towards their utilisation as scale-down models (SDMs), capable to operate under good manufacturing practices (GMP) at the larger scales during late-phase process development (Alves et al., 2015; Sandner et al., 2019; Rotondi et al., 2021). In addition, the perspective of automated operation, increases the ease of handling while reducing human error and enabling parallel reactor runs when different conditions need to be screened. Presently, there are several small scale bioreactor systems that are commercially available, both in stainless steel or glass format and single-use configurations, with volumes ranging from 10s-100s mL, the most popular of which are the ambr® (Sartorius),
DASBOX (Eppendorf) and Mobius® CellReady (Millipore) (Nienow, Scott, et al., 2013; Rafiq et al., 2017; Rotondi et al., 2021).

The ambr® is one of the most utilised small scale bioreactors both for biological screening and engineering studies. It is available at 15 mL and 250 mL sizes and is equipped with addition lines for acid, base, antifoam and media along with sensors for process control. In its internals probes for temperature and pH are included as well as a sparger (depending on the volume) while depending on the application, ambr® can be equipped with baffles and different impeller types in single or dual configurations (Warr, 2020).

Several studies have focused on the characterisation of the ambr® platform, either for the evaluation of its performance for cell culture applications or for the understanding of engineering features and flow elements. Nienow et al. (2013) used the ambr®15 (15 mL) for the cultivation of mammalian CHO cells and reported consistent results when comparing to larger scale vessels (~5 L) with respect to operating variables including dissolved oxygen, pH, and cell growth. Similarly Hsu et al. (2012) used the ambr®15 for mammalian CHO cell culture in a fed-batch mode and compared its performance with shake flasks and vessels with volumes of 2 L. They highlighted the reliability of the ambr®15 to produce similar results to the 2L scale in terms of cell viability, product quality and metabolite concentration. In both studies, comparison of the process performance with shake flasks led to lower cell viabilities in the latter, highlighting the outperformance of bioreactors at mL-scale to efficiently mimic large scale operations and effectively optimise bioreactor performance across scales (Hsu et al., 2012; Nienow, Rielly, et al., 2013b).

To address the limited number of available SDMs for perfusion processes, Tregidgo et al. (2021) developed a custom made 250 mL SDM which has been successfully used in both fed-batch and perfusion cultures of antibody producing CHO cells. Data from this study showed a six-fold increase in viable cell densities when transitioning from fed-batch to perfusion culture by using this small scale reactor. In the same work, the cell culture process in perfusion mode has been scaled-up to 5 L based on constant power per unit volume ($P/V$), where comparable performance was reported with respect to cell growth and production yield (Tregidgo, 2021).

Considering that small scale reactors are usually designed to optimise mixing efficiency and flow dynamics performance - which is particularly important for perfusion studies where nutrient availability should be secured to accommodate the operation of high cell densities -
dissimilarities in geometry and operating flow regime are inevitable and the discrepancies grow even more as the vessel volume becomes smaller (Nienow, Rielly, et al., 2013b). For example, the 15 mL ambr®15 used in mammalian cell and human mesenchymal stem cell cultures has a rectangular shape which makes it significantly different from a cylindrical vessel used in pilot and production scales (Rafiq et al., 2017). Similarly, the blade thickness over impeller diameter ratio is often higher in scale-down reactors than in larger scale due to fabrication constraints arising from the small reactor volume (Rutherford et al., 1996). In addition, volume reduction leads to discrepancies with respect to the flow regime between small and larger scale systems. For instance, a comparison of the ambr®15 with a 5 L vessel based on equal tip speeds resulted in Reynolds numbers (Re) of ~2,600 and ~12,000 for the 15 mL and the 5 L reactor, respectively (Nienow, Rielly, et al., 2013b).

The aforementioned studies underline the challenges when using small scale equipment and highlight the need for extensive and systematic characterisation of the engineering features that govern the mixing and scaling processes which greatly impact bioprocess performance.

1.2.4 Engineering characterisation and flow dynamics studies in STRs

In the context of optimising bioprocess performance to develop robust perfusion strategies and maximise mixing efficiency, the use of small scale bioreactors and the development of new ones is expanding (Nienow, Rielly, et al., 2013a; Xu et al., 2017; Rotondi et al., 2021; Tregidgo, 2021). A few studies have examined engineering aspects in the ambr®15 (15 mL) (Nienow, Rielly, et al., 2013a) and in the ambr®250 (250 mL) (Xu et al., 2017) focusing on characterisation of mixing time and oxygen transfer coefficient (k_La) and compared those to larger scales. For the ambr®15, Nienow et al. (2013) reported lower mixing times comparing to pilot scale vessels and suggested that due to the low liquid volume, oxygen transfer was favoured by the contribution of surface aeration to a greater extent than in a 5 L vessel leading to higher k_La values (Nienow, Rielly, et al., 2013a). Focusing on the ambr®250, Xu et al. (2017) investigated the oxygen transfer and compared the results with corresponding data at 5 and 250 L scale. They found that, contrary to the ambr®15, oxygen transfer through the head space was lower and less effective than that estimated with increasing sparging rates and noted that, due to different sparger configurations among the ambr®250, 5 and 250 L vessels, the former required higher sparging rates to achieve k_La comparable to the larger reactors (i.e. open pipe sparger in ambr®250 versus open pipe sparger in 5-L vessel) (Xu et al., 2017).
Studies dedicated to the in-depth engineering characterisation of SDMs, including characterisation of hydrodynamics and scaling parameters, are limited. Some of those investigations are conducted through experimental fluid dynamics (Odeleye et al., 2014; Wyrobnik et al., 2021) and others by using CFD (Nienow, Scott, et al., 2013; Collignon et al., 2016; Rotondi et al., 2021) tools. In the following sections of this chapter (Sections 1.2.4.1 – 1.2.4.6) the published contributions on flow dynamics in small scale systems will be described alongside principal findings in flow dynamics (experimental and computational) in standardised stirred tank reactors (STRs), including flow patterns, velocity and turbulence distribution and evaluation of macroinstabilities, in order to provide a complete overview of the advances, methodologies and outcomes in the field.

1.2.4.1 Experimental flow dynamics

The flow fields in mixing tanks are characterised by increased complexity due to their three dimensional nature and development of turbulence impacting the mean and periodic flow characteristics (Rutherford et al., 1996). In the past several experimental methodologies have been used to study the flow field in STRs, a detailed review of which is given by Mavros, (2001). The most commonly used methodologies to experimentally characterise the flow field in stirred vessels are Laser Doppler Anemometry (LDA) (Yianneskis, Popiolek and Whitelaw, 1987; Kresta and Wood, 1993; Stoots and Calabrese, 1995; Derksen, Doelman and Van Den Akker, 1999; Ducci and Yianneskis, 2006; Bouremel, Yianneskis and Ducci, 2009b) and Particle Image Velocimetry (PIV) (Escudié and Liné, 2003; Escudié, Bouyer and Liné, 2004; Zhang et al., 2017; de Lamotte et al., 2018b, 2018a).

Yianneskis et al. (1987) investigated with LDA the flow field inside a stirred vessel equipped with a Rushton Turbine (RT) at Re=48,000 and several geometric ratios (CDT =0.25-0.5 and DI/DT=0.3-0.5) and reported maximum values of the mean radial and axial velocities to be \( \sim 0.7u_{\text{tip}} \) and \( \sim 0.25u_{\text{tip}} \) respectively in correspondence of the middle of the impeller blade, decreasing at increasing clearance to impeller diameter ratio (CDT) (Yianneskis, Popiolek and Whitelaw, 1987). Similar radial velocities were reported by Steiros et al. (2017) who used PIV to experimentally investigate the flow field produced by a four-blade Flat Blade Turbine (FBT) at similar C/DT =0.5 and Re~150,000 in a prismatic STR. Phase resolved data indicated that maximum radial velocities in proximity to the blades reached \( \sim 0.65u_{\text{tip}} \) and \( \sim 0.45u_{\text{tip}} \) at 30° and 45° phase angles respectively (Steiros et al., 2017a). Kresta and Wood (1993) studied the flow in a vessel stirred with a 45° PBT via LDA by using two impeller clearances (C/DT=0.25, /DT=0.5) and two impeller diameters (DI/DT=0.25, DT/DT=0.3) in the turbulent flow regime. In
their work, they underlined the dependency of the flow discharge stream in the proximity to the tank bottom by observing a transition from single to double circulation loops with increasing $C/D_T$. The intensity of the secondary circulation loop decreased with decreasing impeller diameter due to a reduction in the measured radial velocity (Kresta and Wood, 1993).

While velocity field has been thoroughly examined and characterised for various impeller configurations, the understanding of turbulence is still evolving as it is associated with challenges encountered in a variety of industrial applications. Derksen et al. (1999) discussed the field of velocity and turbulence in a radially agitated vessel via 3D LDA. They reported that the anisotropic nature of turbulence drops with decreasing intensity of the trailing vortices and suggested that in areas away from the impeller region the isotropic assumption becomes valid (Derksen, Doelman and Van Den Akker, 1999). Moreover, thorough investigation of turbulence was conducted by Escudié et al. (2003) through implementation of flow decomposition and examination of the energy exchange occurring among the mean, periodic and turbulent flow components after conducting PIV measurements in a STR equipped with a RT. Near the impeller blade, periodic fluctuations were reported to be higher than the random fluctuations following a decreasing trend with increasing radial distance due to energy exchange between the periodic and the turbulent motions (source-sink terms respectively) leading to an increase of the latter (Escudié and Liné, 2003). Energy dissipation rate ($\epsilon$) has been experimentally examined by Baldi and Yianneskis (2004) via 2D and 3D PIV in a vessel agitated with a RT who measured dimensionless $\epsilon$ values ($\epsilon/N^3D_T^2$) from 5 to 10 when $15,000<Re<40,000$ (Baldi and Yianneskis, 2004). Ducci et al. (2005) improved the aforementioned measurements by using the higher resolution LDA technique in a similar vessel and estimated 9 out of 12 terms and provided a maximum value of $\epsilon = 12N^3D_T^2$ located at a similar point ($r/D_T=0.22$) in proximity to the impeller blade (Ducci and Yianneskis, 2005a).

With respect to different radial impellers, Dong et al. (1994) studied the hydrodynamics in an unbaffled vessel of smaller volume (1 L) equipped with an eight-blade FBT focusing on the impact of $Re$ and impeller clearance of mean velocity and turbulence distributions. In their work, it was indicated that at $C/D_T\sim0.5$ mean axial, radial and tangential velocities and average kinetic energy were not impacted within $3,300<Re<4,900$. Indicative results corresponded to $u_x\sim0.15u_{tip}$, $u_r\sim0.2u_{tip}$, and $u_\theta\sim0.6u_{tip}$ for axial, radial and tangential velocities respectively and $k\sim0.05u_{tip}^2$ for kinetic energy. Following the trends described previously for the disk turbines, decrease of clearance to tank diameter ratio (from $C/D_T\sim0.5$
to $C/D_T \sim 0.3$) led to a 5-10\% increase of mean velocity and turbulent components while inducing a downward inclination of the impeller stream resulting in a size reduction of the lower circulation loop (Dong, Johansen and Engh, 1994).

In the context of SDMs for the production of mAbs from CHO cell culture in suspension, Odeleye et al. (2014) studied experimentally via PIV the flow dynamics in an unbaffled 3 L Mobius® CellReady (Millipore) bioreactor equipped with a marine impeller ($C/D_T \sim 0.3$), for a range of impeller speeds corresponding to $Re \sim 8,700$-$38,000$. At $Re \sim 20,000$ results showed an upward inclination of the impeller jet, estimated $\sim 26^\circ$ from the impeller centreline. They reported good agreement of mean $(|u| \sim 0.25u_{tip})$ and turbulent $(u'/u_{tip} \sim 0.11$ and $u'/z/u_{tip} \sim 0.15)$ velocities with literature data corresponding to standardised vessels agitated with up-pumping 45° PBT and 10° 3-blade hydrofoil impellers. Comparison of results across different Reynolds numbers ($8,700 < Re < 38,000$) showed that the velocity scaled linearly with $u_{tip}$ indicating that the flow characteristics remained consistent across the $Re$ range and that the bioreactor operated at the turbulent flow regime even at the lower $Re \sim 8,700$ limit. Likewise, the ensemble average turbulent kinetic energy calculated in proximity to the impeller swept volume was reported to be stable across the range of $Re$ ($k' \sim 0.015u_{tip}^2$) but obtained lower values when compared to results produced by similar impellers in larger tank volumes (5-10 L) (Odeleye et al., 2014). In another work for similar application, Wyrobnik et al. (2021) studied via PIV the flow produced by a marine impeller, a 45° 3-Blade segment (3BS) and a novel Bach impeller in the commercial unbaffled 1 L Univessel® (Sartorius) at $Re \sim 2,900$. They reported maximum velocity magnitude $|u| \sim 0.25u_{tip}$ for the 3BS and the marine impeller at $C/D_T \sim 0.3$. Data for average kinetic energy were in the range of $k \sim (0.015-0.02)u_{tip}^2$ but for both velocity and turbulence maximum regions were expanded over a higher fluid volume in proximity to 3BS impeller. For the novel Bach impeller flow studies indicated minimum sensitivity of the impeller clearance on the impeller performance and lower shear compared to conventional designs (i.e. marine and 3BS impellers) underlining the suitability of its impeller for animal cell cultures (Wyrobnik et al., 2021).

1.2.4.2 Computational fluid dynamics

Computational Fluid Dynamics (CFD) has gained more attention during the last 20 years as it allows the investigation of multiple parameters for the prediction of flow patterns in single and multiphase systems (Sarkar et al., 2016; Tamburini et al., 2018). Initial works focused on modelling the entire flow field by using Reynolds Averaged Navier-Stokes (RANS) equations as a way to acceptably predict turbulent flows in STRs; but reported lack of accuracy when
estimating turbulent quantities since the entire range of turbulent length scales is modelled (Yeoh, Papadakis and Yianneskis, 2004; Delafosse et al., 2008; Joshi et al., 2011a; M Coroneo et al., 2011). To better resolve the hydrodynamics in stirred reactors and accurately examine the evolution of velocity gradients and turbulence, Scale Resolving Simulations (SRS) were implemented including Scale Adaptive Simulations (SAS) (Egorov et al., 2010; Zamiri and Chung, 2018), Detached Eddy Simulations (DES) (Gimbun et al., 2012; Chara et al., 2016a) and Large Eddy Simulations (LES) (Hartmann et al., 2004; Delafosse et al., 2008; Collignon et al., 2016). The first two operate as hybrid models, switching between RANS and LES approach depending on the turbulence scale determined near the flow boundaries. Therefore, the smaller eddies are filtered out during the averaging process and the grid requirements are less demanding leading to lower computational cost (Egorov et al., 2010; Wang et al., 2017; Zamiri and Chung, 2018). In LES, the large anisotropic eddies are resolved and only a small part of the flow is modelled which corresponds to the smaller eddies of the energy cascade. The resolution of LES and the cut-off for the initiation of modelling is determined by the grid size and is estimated based on the order of Taylor microscale, therefore the grid requirements are high (Hartmann et al., 2004; Delafosse et al., 2008; Collignon et al., 2016; Liné, 2016). RANS and SRS can be validated with Direct Numerical Simulations (DNS), in which the entire range of turbulent length scales are fully resolved without the use of any modelling. DNS could potentially replace the experimental procedures for the characterisation of hydrodynamics but even though DNS have been implemented for flow investigation at low and intermediate Re their use is limited due to their high computational cost (Gillissen and den Akker, 2012; Başbuğ, Papadakis and Vassilicos, 2017).

An overview of the performance of CFD models operating under RANS approaches is given by Joshi et al. (2011), who conducted CFD simulations in single phase flow STRs and compared the results to LDA data. They detailed strengths and weaknesses associated with k-ε models and the Reynolds Stress Model (RSM) after examining the individual turbulence terms (i.e. dissipation, transport and diffusion) using both steady state and transient approximations and underlined the consistent underprediction of turbulence quantities in proximity to the impeller blades. Bakker and van den Akker (1994) and Aubin et al. (2004) studied the performance of RANS models focusing on k-ε Standard, k-ε RNG and Reynolds Stress Model (RSM) for both axial and radial impellers. They reported good agreement between CFD and experimental data with respect to the computed velocities but discussed the better performance of RSM on turbulence prediction (Aubin, Fletcher and Xuereb, 2004a). In another
work Aubin et al. (2006) used RANS equations to examine the flow field produced by an axial impeller in a bioreactor operating in continuous mode. In their work they characterised the hydrodynamics generated in both up- and down-pumping modes while altering the flowrate of the inlet. They reported that when an up-pumping impeller was used, circulation loops were maintained unaffected by the presence of the inlet feed resulting in more robust flow patterns and underlined minimal impact of tangential flow in the feeding jet (Aubin et al., 2006). Montante et al. (2001) used k-ε Standard, RNG and RSM operating under RANS approach to study the flow field in a stirred vessel equipped with a RT at different clearances. They reported a good performance of CFD simulations in predicting the velocity field across the different conditions, including the transition from double to single circulation loops, evident at low impeller clearance (Montante et al., 2001). In a later study, Coroneo et al. (2011) and Deglon et al. (2006) discussed technical issues around CFD RANS modelling and assessed the impact of discretisation schemes and grid density on the overall simulation performance. They suggested that the use of high order discretisation schemes leads to better agreement between simulation and experiments especially for coarser grids while the use of very fine grids significantly improved predictions of turbulent kinetic energy in comparison to experimental data (Deglon and Meyer, 2006; M. Coroneo et al., 2011; Gimbun et al., 2012).

A wide range of studies focused on the characterisation of Scale Resolved Simulations (SRS). Hartmann et al. (2004) assessed LES performance by using two different subgrid scale models, the Smagorinsky and the Voke model. Both models led to similar results and were in agreement with experimental data for velocity and turbulence and underlined the better performance of LES compared to RANS approaches (Hartmann et al., 2004). Delafosse et al. (2008) compared LES and Unsteady RANS (URANS) simulations for the flow generated in a STR equipped with a RT. Due to the capability of LES to resolve the instantaneous velocity field it was reported that the model acceptably predicted the periodic and turbulence contributions in the flow which led to good representation of the trailing vortices compared to experiments. In the same work, estimation of $\varepsilon$ showed agreement between LES predictions and available experimental data and outperformed URANS which could estimate the order of magnitude of energy dissipation rate but predictions failed to reproduce its distribution (Delafosse et al., 2008, 2009). Gimbun et al. (2012) and Zamiri et al. (2018) used DES and SAS respectively to simulate the flow in baffled radially agitated STRs and reported agreement of those models with experimental and LES data in terms of the predicted velocity, turbulence and evolution of vortical structures. The good flow predictions in combination with lower
requirements for spatial resolution characterising those hybrid models underlined their suitability for flow analysis at industrial scales at relatively low computational cost (Gimbun et al., 2012; Zamiri and Chung, 2018).

Different flow characteristics coming from CFD datasets have been discussed and compared across the various models, but the accuracy of CFD simulations has been assessed and validated in larger scale systems (V=10-70 L) and vessels with standardised geometries. Conversely, for small scale bioreactors the data availability on flow at the corresponding operating conditions (i.e., lower Re, presence of baffles and probes) is limited.

Kaiser et al. (2011), used RANS simulations to study spatial distribution of flow patterns in the unbaffled 3 L Mobius® CellReady (Millipore) equipped with a marine impeller at Re~10^4. In their work, they reported maximum velocity magnitude ~0.4u_{tip} in proximity to the impeller and underlined three-fold decrease in velocity magnitude above the impeller swept volume leading to poor homogeneity in gas dispersion in the upper tank region (Kaiser, Regine and Dieter, 2011). It is interesting to note that for similar Re, the maximum |u| presented in this study was ~60% higher than the value measured experimentally by (Odeleye et al., 2014) and this can be attributed to the presence of two probes which were considered in the former, which contributed to the increase of velocity magnitude in a way similar to baffles (Kaiser, Regine and Dieter, 2011).

At 15 mL-scale, Nienow et al. (2013) used the RANS approach to estimate the velocity and turbulence distributions in the ambr®15 equipped with a two-blade 45° axial up-pumping impeller, resembling an elephant ear impeller, and operated at Re~3,200. High velocity and turbulence levels were found in close proximity to the impeller, with |u|~0.5u_{tip} whereas at the bulk regions velocity magnitude dropped one order of magnitude (Nienow, Rielly, et al., 2013a). Results were in agreement with those measured experimentally for a ~2.6 L baffled vessel mixed with an elephant ear impeller operating at Re~22,000 (Zhu et al., 2009). To examine turbulence Nienow et al. (2013) extracted the energy dissipation rate from the RANS dataset which was reported to be one order of magnitude higher than the suggested ε in which animal cell cultures are known to operate without the occurrence of cell death (~0.2 W/kg in ambr®15 versus 0.02 W/kg in pilot and commercial scales); though a minimum impact of shear on culture performance was observed which was attributed to the gradual adaptability of cells to the culture environment (Nienow, Rielly, et al., 2013b; Nienow, Scott, et al., 2013). Collignon et al. (2016) used RANS and LES to simulate the flow in a 200 mL small scale bioreactor and assessed the impact of multiple impeller configurations, including elephant ear
impeller, RT and marine impeller, on flow at $Re=2,613$. They reported good agreement between the two CFD approaches with respect to velocity distribution whereas for $\varepsilon$ while RANS correctly predicted its magnitude, it failed to accurately reproduce its spatial distribution. Indicative values obtained for velocity magnitude corresponded to ~$0.5u_{tip}$ for the elephant ear and marine impellers and ~$0.6u_{tip}$ for the RT. Velocity results were in agreement with Rotondi et al., (2021), who investigated the flow field via RANS simulations in the ambr®250 (250 mL) equipped with a similar elephant ear impeller at $Re \approx 2,500$.

**1.2.4.3 Characterisation of trailing vortices**

In agitated systems conversion of mechanical energy into momentum is significantly impacted by the impeller configuration, which is critical for the hydrodynamic and mixing characteristics developed (Martínez-Delgadillo et al., 2019). In this regard, several studies have been dedicated to the computational and/or experimental investigation of standard and novel impeller designs (Kim and Manning, 1964; Steiros et al., 2017a; Martínez-Delgadillo et al., 2019; Hoseini et al., 2021; Wyrobnik et al., 2021) by mainly focusing on the trailing vortices generated by each impeller as the development and stability of the latter can control power consumption, dispersion and coalescence in gas-liquid mixing and ultimately determine reactor performance (Van’t Riet, Bruijn and Smith, 1976).

Several approaches have been reported in the literature for the localisation and visualisation of the trailing vortices. Riet and Smith (1975) identified the trailing vortices produced by a RT based on the position where phase resolved axial velocity is zero and found that for $Re>5,000$ the trajectory of the vortex axis was independent of $Re$ (Riet and Smith, 1975). This method has been also used in other works for characterisation of the position of the vortex axis (Yianneskis, Popiolek and Whitelaw, 1987; Lee and Yianneskis, 1998; Escudié, Bouyer and Liné, 2004). Moreover, trailing vortices can be visualised based on phase resolved vorticity magnitude. Indicative works implementing this method include Delafosse et al. (2009), Chara et al. (2019) and Martinez-Delgadillo et al. (2019) who computationally examined the trailing vortex evolution according to the hydrodynamics produced by a RT (Delafosse et al., 2009; Chara et al., 2016b) and a modified (grooved) 45° PBT, (Martínez-Delgadillo et al., 2019). A second visualisation method of the trailing vortices was suggested by Jeong and Hussain, (1995) and is based on the calculation of the eigenvalues of the symmetric ($S$) and antisymmetric ($\Omega$) part of the velocity gradient tensor ($S^2 + \Omega^2$). The centre of the trailing vortex is determined from the location of the highest absolute value of the second negative eigenvalue of $S^2 + \Omega^2$. The studies of Escudié et al. (2004) and Başbuğ et al. (2017) are examples
of published works illustrating trailing vortex structure either experimentally or computationally by two radial impellers, a standard RT (Escudié, Bouyer and Liné, 2004) and a four-blade FBT (Başbuğ, Papadakis and Vassilicos, 2017). According to the work of Escudié et al. (2004) this method is the most complex and computationally demanding but results in accurate data as it is based on the localisation of the pressure minimum characterising the trailing vortex core (Escudié, Bouyer and Liné, 2004; Delafosse et al., 2009). A third method to visualise the trailing vortices is based on producing the isosurface of the second invariant of the velocity gradient tensor ($S^2 + \Omega^2$, where $S$ and $\Omega$ correspond to strain rate and vorticity tensor respectively). This method ($Q_{cr}$) indicates the local balance between strain rate and vorticity magnitude according to which the vortices are defined from the regions where the vorticity is higher than the strain rate (Zamiri and Chung, 2018). Arosemena et al. (2022) used 3D computational data to visualise the trailing vortices through this technique in a radially mixed 10 L STR at high Re and characterised the vortices as spherical structures in areas close to the level of the impeller (Arosemena, Ali and Solsvik, 2022).

The most studied flow in STRs is the one developed by the RT as it is an impeller used in the vast majority of industrial processes. RT produces a highly energetic radial flow and it has been estimated that almost 70% of the energy supplied to the impeller is dissipated in the discharge stream in its vicinity (Cutter, 1966; Rutherford et al., 1996). Riet and Smith (1973) were the first to examine the structure of the trailing vortex pair formed behind the blades of a RT and mentioned that the centrifugal acceleration of the trailing vortices caused by impeller rotation would influence the liquid-liquid and solid-liquid dispersions in multiphase systems, as trailing vortices act as cavities for gas bubbles or solid particles. They related the trailing vortex formation and propagation with high shear levels, mentioning that the shear rates developed in its vicinity can be ten-fold higher than average values reported in the literature (Riet and Smith, 1975; Riet, Bruijn and Smith, 1976). Yianneskis et al. (1987) experimentally investigated the flow dynamics and trailing vortex formation in a reactor mixed with a RT, using various impeller clearances ($0.25<C/D_r<0.5$) and diameters ($0.25<D_i/D_r<0.5$). They found an increasing inclination of the impeller stream ranging from 2.5° to 7.5° with decreasing $C/D_r$ and discussed that the trailing vortex trajectory can be approximated by $r = 30 + 4.93\varphi$, where $r$ is the radial displacement and $\varphi$ is the impeller phase angle. Moreover, similarly to previous works (Cutter, 1966; Riet and Smith, 1975; Rutherford et al., 1996), they correlated the flow in proximity to the trailing vortex core, with maximum turbulence levels ($k'\approx0.4u_{tip}^2$), approximately 50% higher than in the areas in the bulk of the tank (Yianneskis,
Popiolek and Whitelaw, 1987). In terms of vortex geometry, Escudié et al. (2004) visualised the trailing vortices based on the Jeong and Hussain, (1995) method, described previously, and reported that the vortex diameter was approximately equal to half of the blade width (Escudié, Bouyer and Liné, 2004).

The intention to further elucidate trailing vortex formation in combination with the aforementioned techniques for vortex identification, led to the development of 3D schematic representations of the trailing vortices generated by RTs. With respect to FBTs, Winardi and Nagase (1994) studied the trailing vortex structure and provided an illustration of their shape. According to their results, even though the properties of trailing vortices emanating from both types of radial impellers are similar, they differ in terms of the separation mechanisms from the impeller blades (cf. Fig. 1.1) (Winardi and Nagase, 1994). In RTs and in baffled vessels, due to the presence of the disk the trailing vortex separation occurs at the inner part (leading edge) of the blade, but in FBTs where the disk is absent and blades are extended up to the impeller shaft, the vortex separation is transferred closer to the impeller hub preventing the leading edge separation (Nienow and Wisdom, 1974; Winardi and Nagase, 1994; Steiros et al., 2017a). The transition of the separation point in FBTs is associated with the transition of the pressure minimum localised at the vortex core and justifies the reduction of power number ($P_0$) observed between FBTs and RTs (for fully baffled tanks, $P_0$~4 and ~5 for FBTs and RTs respectively according to (Bates, Fondy and Corpstein, 1963)).

Due to the similarity in the main flow characteristics between FBTs and RTs, most of the hydrodynamics characterisation and validation of new methodologies in standardised radial flows, are conducted having the RT as a baseline. Moreover, previous studies investigating trailing vortices from FBT confirm the similarity between those two radial impeller types. For example, Mochizuki et al. (2007) used LDA to examine the trailing vortices emanating from a four-blade FBT ($C=0.5D_T$) in a fully baffled tank with $V=92$ L and at $Re$~$10^5$. They reported the existence of two symmetric trailing vortices with an ellipsoid shape and diameter in the $z$ direction which was half the blade width. In the same work, comparison of the trailing vortex trajectory with results from standard RTs, indicated similarity between the two impeller types in terms of vortex path and expansion (Mochizuki et al., 2007). Steiros et al. (2017) used PIV and visualised the trailing vortices produced by a four-blade FBT ($C=0.5D_T$) based on vorticity magnitude at $Re$~150,000 in a vessel with octagonal circumference. They observed two distinctive trailing vortices moving in parallel and associated with the local maximum turbulence intensity ~$0.45u_{tip}$. Similarly Başbuğ et al. (2017) used DNS to simulate the flow
field produced by a four-blade FBT ($C=0.5D_T$) in an unbaffled vessel at $Re\approx 1,500$. In their work, the two vortices propagated normal to the blade and obtained a curved profile which was tilted upwards or downwards due to impingement of the radial flow at the vessel walls (Başbuğ, Papadakis and Vassilicos, 2017).

Investigation of trailing vortices has been conducted not only for radial but also for axial and novel impeller configurations (Kresta and Wood, 1993; Ng et al., 1998; Bugay, Escudié and Line, 2002). At a smaller scale reactor, Odeleye et al. (2014) studied, the trajectory of the trailing vortices in the 3 L Mobius® CellReady (Millipore) equipped with a marine impeller based on the calculation of the maximum vorticity magnitude. It was found that the trailing vortex cores moved similarly within a $Re$ range of 21,700 – 38,000 but underlined that the radial path travelled by the trailing vortices was shorter compared to similar axial impellers due to high impeller to tank diameter ratio (Odeleye et al., 2014). In larger tanks ($V=5\text{-}10 \text{ L}$) Başbuğ et al. (2018) and Martínez et al. (2019) used CFD to investigate vortical structures in fractal and grooved impellers respectively and indicated that the existence of notches and groove valleys split the continuous and robust vortical structures into smaller vortices suggesting their suitability in applications where long mixing is required (Başbuğ, Papadakis and Vassilicos, 2018b; Martínez-Delgadillo et al., 2019).

Considering the impact of trailing vortices on flow patterns and turbulence, their influence on mixing processes has been investigated by several research works (Assirelli et al., 2005; Bouremel, Yianneskis and Ducci, 2009a; Başbuğ, Papadakis and Vassilicos, 2018a). Aiming to analyse vorticity and strain dynamics in a STR equipped with a RT, Bouremel et al. (2009) investigated the deformation forces existing in proximity to the trailing vortices based on experimental LDA data. In their work they found high stretching and compression forces in close proximity to the vortex region and suggested that this information, coupled with interfacial tension, could shed light on the dispersion dynamics of multiphase systems (Bouremel, Yianneskis and Ducci, 2009a). Assirelli et al. (2005) reported a seven-fold increase in micromixing efficiency after experimental studies on vessel hydrodynamics generated by a RT. In their work they related the optimal position of injection points to areas characterised with the highest energy dissipation of the system which coincided with the trailing vortex cores (Assirelli et al., 2005). More recently Başbuğ et al. (2018) after computationally investigating the flow produced by $45^\circ$ PBT with fractal blades, reported a 12% decrease in mixing time due to increased radial velocity and intensified stretching and folding of the flow.
in the vicinity of the trailing vortices promoted by the fractal blades (Başbuğ, Papadakis and Vassilicos, 2018a).

1.2.4.4 The impact of baffles
To improve mixing performance and prevent solid body rotation, STRs operating with fluids of low viscosity are usually equipped with baffles. Still, there is a variety of applications in which the use of UB tanks is preferred, including crystallisation and precipitation processes, where baffle presence may promote particle attrition (Rousseaux, Muhr and Plasari, 2001; Scargiali et al., 2014). UB vessels are also used in food and pharmaceutical industries where sterility and tank cleanness is of major importance, as well as in laminar mixing of high viscosity fluids, to prevent dead zones from forming in the proximity of the baffles (Lamberto et al., 1996; Scargiali et al., 2014). Several works have mentioned the advantage unbaffled over baffled vessels for efficient suspension and oxygen transfer mechanisms at lower power requirements (Brucato et al., 2010; Brucato, Busciglio and Scargiali, 2017). Scargiali et al. (2014) investigated mass transfer characteristics in UB stirred bioreactors when equipped with various impeller types and reported that an impeller rotation at the super-critical flow regime (see (Scargiali et al., 2017)) could achieve a mass transfer performance equivalent to a sparged system with reduced shear levels (Scargiali et al., 2014).

Computational characterisation of turbulent flow in unbaffled vessels is challenging due to the surface deformation caused by the absence of baffles. Ciofalo et al. (1996) studied the flow in closed UB vessel equipped with radial impellers including an eight-blade FBT and a RT. They observed incapability of k-ε models to reproduce the flow due to deviations in tangential velocity and loss of secondary motions caused by the rigid body rotation. The results were improved when Reynolds stress transport equations were directly resolved through a differential turbulence stress model and when surface deformation equations were incorporated in flow solution (Nagata, 1975; Ciofalo et al., 1996). Alcamo et al. (2005) used CFD LES to model the radial flow produced by a RT in a closed UB vessel and reported good agreement between simulations and PIV data for velocity distributions, turbulence predictions and trailing vortex representation (Alcamo et al., 2005). Płusa et al. (2021) characterised the vessel hydrodynamics in open and closed STRs both in the presence and absence of baffles via CFD RANS and LDA, and the agreement between simulations and experiments was acceptable near the impeller for both vessel configurations, suggesting that the simulation of the free surface can be omitted with 80% reduction in computation time (Płusa et al., 2021).
Comparison between baffled and unbaffled tanks along with flow dynamics and mixing characterisation of STR performance with different baffle designs has been reported in the past (Chapple and Kresta, 1994; Lu, Wu and Ju, 1997; Roussinova, Grgic and Kresta, 2000; Atibeni and Gao, 2013; Luo et al., 2015). Atibeni et al. (2013) explored experimentally suspension mechanisms in an STR equipped with up-triangular and down-triangular baffles and reported that the performance of the latter outweighed the standard baffle design exhibiting lower power consumption and critical agitation speed for particle suspension (Atibeni and Gao, 2013). In previous years Lu et al. (1997) studied the dependency of mixing time on baffle number and size and mentioned that excessive baffling had an adverse effect on mixing efficiency (Lu, Wu and Ju, 1997). Fan et al. (2021) studied experimentally the flow dynamics from a 45° PBT in unbaffled and baffled configurations and reported increased mean velocity magnitude and turbulence levels in the latter due to the greater resistance induced by the baffle presence. Moreover, they mentioned that the presence of baffles will favour axial or radial velocity components depending on the initial origin of the flow generated by the impeller, i.e. radial velocity will become higher in baffled tanks agitated with radial impellers and axial velocities will become higher in baffled tanks stirred with axial impellers (Fan et al., 2021). In the same context Ammar et al. (2011) reported increasing radial and axial velocities with increasing baffle length after presenting CFD RANS simulation data for a STR equipped with a RT. It was also reported that the baffled length determined the intensity of flow circumferential motion inside the vessel which appeared to significantly decrease with increasing baffle length (Ammar et al., 2011). Foukrac and Ameur (2019) investigated the impact of baffle curvature and position on flow patterns in a radially agitated vessel and suggested that curved baffles increased the intensity of the radial jet compared to the standard baffle configurations due to strong interaction between curved baffles and impeller discharged stream. With respect to the position of the baffles they reported that when the curved baffles were located at the middle of the tank height, the flow circulation loops were further intensified which is promising for better mixing performance (Foukrach and Ameur, 2019).

In smaller scale systems, there are limited published data analysing the impact of baffles on flow. However, previous works have studied small scale bioreactors in the presence of probes mentioning that they can act in an equivalent way to baffles. Collignon et al. (2016) simulated via LES the average velocity field in a 200 mL STR equipped with two probes, a sampling tube and elephant ear impeller at down-pumping mode ($Re=2,613$) and compared the average in-
plane velocity in the bulk and in proximity to the bioreactor internals. According to this work, the various axial planes illustrated similar flow patterns in terms of the number and the location of the generated circulations loops, though the size of those loops was dependent on the plane distance from the internals, with larger loops obtained away from the probes.

Vertical profiles of the average velocity magnitude across the different planes showed a slight increase (~10%) at the planes near the probes and no surface vortexing was observed, confirming that the presence of those internals disrupted the intense solid body rotation in a way similar to baffles (Collignon et al., 2016). Aiming at comparing the average velocity field with and without baffles, Rotondi et al. (2021) simulated via RANS the average velocity magnitude in the ambr®250 equipped with an elephant ear impeller, two probes (for temperature and pH monitoring) and a sparger, at Re~2,500 and discussed that the resulting flow patterns were similar with and without the presence of baffles and resembled larger baffled STRs operating with similar impeller configurations. They implied that the three internals (pH, temperature probes and sparger) were enough to provide the flow with advantages of baffling (Rotondi et al., 2021). Similar conclusion was reached by Nienow et al. (2013) for the ambr®15 (15 mL), equipped with a sparger and operated at Re~3,200, who comparing average velocity data obtained via RANS simulations with larger baffled vessels mixed with similar impellers, they reported resemblance in the resulting flow field inferring that in a smaller reactor volume, such as the ambr®15, a single internal may act equivalently to baffles in a fully baffled STR at larger scales (Nienow, Rielly, et al., 2013a).

### 1.2.4.5 Impact of macroinstabilities on flow dynamics

Traditional studies in STRs have focused on the characterisation of the turbulent flow aiming at understanding the velocity and turbulence patterns. Similarly to the velocity decomposition into mean, periodic and fluctuating components, the frequencies associated with the flow field in stirred vessels can be higher than the blade passage when they are related to the turbulent fluctuations (frequencies up to the Kolmogorov wavenumber); equal or harmonic of the blade passage frequency (BPF), when they are associated with the trailing vortices and periodic motion of the impeller; lower than the blade passage when representing a much lower variation of the mean. The last term is referred to as macroinstability (MI) and shows the presence of flow structures with time scales lower than those associated with the blade passage.

MIs are important components of the macroscopic large scale motion in STRs with potential implications on process performance including mass, heat transfer and mixing but also on the
structural integrity of the vessel (Hasal et al., 2000; Roussinova, Kresta and Weetman, 2003). Ducci and Yianneskis (2007) studied MI vortices (i.e. perturbations of the instantaneous flow field with a certain frequency depending on the operating Re) in a radial flow produced by a RT and identified that tracer insertion along the path of the MI vortices was associated with a decrease in mixing time of ~20-30% (Ducci and Yianneskis, 2007). Roussinova et al. (2002) described the main sources triggering the generation of MIs in STRs which include the impeller jet impingement on the tank walls and bottom and the trailing vortices (Roussinova, Kresta and Weetman, 2003).

MIs can occur in different regions of the working volume in a STR and appear at different frequencies while depending on their amplitude and period can influence turbulence levels. Nikiforaki et al. (2003) discussed different types of microinstabilities characterised by various frequencies and found that MIs can lead to 6-25% increase of turbulence levels after examining a stirred vessel equipped with a PBT at high Re (Nikiforaki et al., 2003). In the same context Roussinova et al. (2000) suggested that MIs can mostly affect turbulence levels when observed within a period of 10 s and have an amplitude >5% of the impeller tip speed (Roussinova, Grgic and Kresta, 2000).

Due to time and spatial resolution constraints most of the published works on flow MIs are based on experimental data. Rutherford et al. (1996) conducted LDA and observed changes in flow patterns in radially agitated vessels with multiple impellers and discussed that depending on the distance between the impellers, the clearance of the lower impeller and the submergence of the top impeller in the working fluid, three stable and four unstable flow patterns were identified. In their work, they found the range of design parameters (i.e. clearance, impeller separation and submergence) in which the stable flow patterns were observed - named as “merging”, “parallel” and “diverging” - while beyond those parameters they detected unstable flow patterns, appearing with a period of ~10 min, which significantly impacted power consumption and increased mixing time (Rutherford et al., 1996). Similarly, Galletti et al. (2005) reported an exchange of flow patterns from single to double circulation loops in a vessel stirred with a RT at lower clearances, based on flow analysis conducted on LDA dataset, while in another work they underlined the MI frequency is dependent on the investigated Re (Galletti et al., 2004; Galletti, Paglianti and Yianneskis, 2005). Winardi and Nagase (1991) experimentally examined the flow pattern around a marine propeller and identified the generation of random flow patterns with lifetimes from 0.5 ms to several minutes, affecting the tangential velocity component and altering the direction of the impeller
stream (Winardi and Nagase, 1991). Meaningful results on the flow stability were reported by Chapple and Kresta (1994), who experimentally explored the effects of design parameters such as impeller type, off-bottom clearance and baffle number. According to their work, the most important parameters to determine flow stability at the impeller stream were impeller diameter, baffle number and interaction between the two (Chapple and Kresta, 1994). Roussinova et al. (2000) studied via LDA the MIs at the impeller stream in a vessel equipped with an assortment of different impeller types (RT, PBT, HE3 and A310) and different number of baffles (two and four) and concluded that reducing the baffle number and impeller diameter triggers more flow instabilities in the case of the RT, rather than in axial impeller systems (Roussinova, Grgic and Kresta, 2000).

Computational modelling has been also used to study MIs in STRs mostly via LES as RANS methods due to their averaging nature, do not account for resolving the instantaneous flow-field and hence such temporal variations (Nikiforaki et al., 2003). Roussinova et al. (2003) used both LDA and CFD LES to study the MIs produced by a 45° PBT in two vessels of different scales. After a simulation time corresponding to 30 impeller revolutions, they adequately resolved and validated the MIs observed experimentally for both bioreactor scales ($f_{MI} \sim 0.6$ Hz or $f_{MI}/N \sim 0.186$). Hartmann et al. (2004) studied the whirlpool type vortex forming around the tank centreline via RANS and LES modelling. They highlighted the accuracy of LES to capture the spatial implications of this MI but stressed the difficulty to capture its time scales as more 100 revolutions were required per run for adequately resolved data (Hartmann, Derksen and van den Akker, 2004).

### 1.2.4.6 Proper Orthogonal decomposition in fluid dynamics

Proper Orthogonal Decomposition (POD) is a statistical technique and is based on identifying the dominant orthogonal modes that can be used to reconstruct the dataset capturing most of the energy contained in the system. POD has been extensively applied in the literature in stirred reactors for the formation of Low Order Models (LOMs) aiming at reducing the dimensionality of the system under investigation, through flow reconstruction only via using the most energetic modes which are representative of the most coherent flow structures (Ducci, Doulgerakis and Yianneskis, 2008; de Lamotte et al., 2018b). Liné et al. used POD to analyse the flow structures occurring in Newtonian and non-Newtonian fluids and broke down the contributions of mean, organised and turbulent motions to the total kinetic energy and energy dissipation rate after correlating the POD eigenvalue spectrum with the kinetic energy corresponding to each mode (Liné et al., 2013; Liné, 2016). De Lamotte et al. (2018)
assessed the eigenvalue spectrum between CFD RANS and PIV data and discussed the agreement between RANS and PIV with respect to the reconstruction of the large coherent periodic flow motions (i.e. trailing vortices) but underlined the weakness of RANS to capture lower energy turbulent fluctuations due to their averaging nature (de Lamotte et al., 2018b).

Mayorga et al. (2021) used CFD RANS data in a stirred vessel equipped with a RT to compare results after applying POD on the individual zones comprising the CFD domain (stationary and rotational) and the entire-global flow domain. They reported that in all cases the first three modes were associated with the mean and dominant periodic flow motions in proximity to the blades and/or baffles while capturing 99.9% of the total variance verifying that were adequate to represent the total flow field (Mayorga, Morchain and Liné, 2022). In a similar way Weheliye et al. (2018) applied POD on experimental flow data for a shaken system and reported that the first two models were associated with the rotational frequency of the shaker table in a similar way that the corresponding frequencies are associated with the blade passage in a STR (Rodriguez et al., 2013; Weheliye et al., 2018).

Due to the fact that POD can be used to extract highly energetic flow structures, it has been used for the investigation of MIs in STRs. Hasal et al. (2000) used POD to detect and quantify MI in a fully baffled STR equipped with a PBT, on velocity data collected via LDA in different Re, covering the laminar, transitional and turbulent flow regime. They reported that the MI was easier to detect in low Re where turbulent chaotic motions are limited and mentioned that it did not scale with impeller rotational speed (Hasal et al., 2000). Ducci et al. (2008) used POD to analyse the impact of MIs on mean flow and vortex formation in transitional and high Re and reported that the existence of MIs led to precession and elongation of the vortex core, more evident at intermediate Re (Ducci, Doulgerakis and Yianneskis, 2008). In a later work, Doulgerakis et al. (2011) assessed the MIs of the flow field produced by a PBT after applying POD in multiple horizontal and vertical planes in the stirred vessel and reported the existence of two types of MIs which are responsible for inducing impeller jet oscillations and a precessional motion around the impeller shaft (Doulgerakis, Yianneskis and Ducci, 2011).

1.2.5 Power consumption and scale-up

Power consumption (or power drawn) \( (P) \) is the amount of energy required per unit of time to generate movement of fluid inside the reactor. Power consumption is strongly dependent on the tank design parameters, baffle presence and configuration, impeller type, sizing and location, fluid characteristics and flow regime (Ghotli et al., 2013). Sánchez Pérez et al. (2006) reported that \( P \) is expected to be proportional to \( \propto N^2 \) and \( \propto N^3 \) in laminar and turbulent flow
regimes, respectively (Sánchez Pérez et al., 2006). Power input can be calculated according to eq. 2.2 and subsequently, power number, $P_0$, can be estimated (see eq. 2.3) which is a dimensionless parameter characteristic of each impeller. Moreover, power input is used as an indirect method to estimate the average levels of shear existing in a reactor as it is directly proportional to the global energy dissipation rate, according to eq. 2.4.

Several techniques have been used for the measurement of the power consumption in stirred vessels including watt meters, calorimeters, dynamometers, torque meters and systems based on strain gauges (Ascanio, Castro and Galindo, 2004). The first techniques used for power input measurements in STRs were based on electrical measurements conducted on the motor by ammeters and watt meters. Although the instrumentation and cost for this method are simple and low, the friction losses are not accounted for and may be high especially for bench scale vessels (losses can reach up to 70% of the total power) (Brown, 1997; Ascanio, Castro and Galindo, 2004). Calorimetric measurement is a high precision technique based on energy balances from which the power dissipated from viscous forces can be determined, though high sensitivity instrumentation is required to ensure the insulation of the system and reduce the energy losses (Carreau, Paris and Guérin, 2009). Operation of dynamometers is based on Newton’s third law: the agitator applies force to the liquid to which the latter presents resistance; the liquid produces a force transmitted to the impeller and motor and the reaction force leads to free rotation of the vessel on its bearings. The advantage of this method is that precision measurements can be carried out considering the losses, making this method appropriate for power measurements in the range 5-15 kW, which are typical for reactors at bench and pilot scales (Nienow and Miles, 1971; Brown, 1997; Ascanio, Castro and Galindo, 2004). Finally, torque meters and strain gauges measure the torque via transducers that correlate the strain generated with the electrical resistance which is proportional to the torque applied (Noltingk, 1989; Brown, 1997; Ascanio, Castro and Galindo, 2004).

Several studies have described the $P_0$ curves for various impeller types and sizes in baffled and unbaffled tanks (Bates, Fondy and Corpstein, 1963; Nienow and Miles, 1971; Bakker et al., 1996). In principle radial flow impellers, such as FBT and RT, are characterised by higher power numbers compared to axial flow impellers, i.e. PBT, marine, 3BS, which renders them a rare option for animal shear sensitive cells (Bates, Fondy and Corpstein, 1963; Nienow and Miles, 1971; Nienow, 2006). In addition, baffle presence in STRs enhances the top to bottom circulation loops generated by radial impellers contributing to an increase in the $P_0$. In this respect, the use of axial impellers is preferred in unbaffled tanks due to the fact that the axial
circulation is promoted with lower power demands. Moreover, depending on the flow type, $P_0$ can be impacted by the impeller diameter and impeller blade thickness. For radial impellers $P_0$ is found to increase with impeller diameter whereas it decreases with increasing blade thickness to impeller diameter ratio (Nienow and Miles, 1971; Kresta and Wood, 1993; Rutherford et al., 1996). The latter might be relevant in small scale systems as due to fabrication constraints the ratio of blade thickness to impeller diameter is higher compared to bench and pilot scale vessels.

The dissipated energy to a fluid is a driving force of mixing but it is also a source of shear that may potentially influence cell viability. The most common criteria used for scale-up in animal cell cultures include oxygen transfer coefficient ($k_L a$), impeller tip speed ($u_{\text{tip}}$) and power per unit volume ($P/V$), but these are qualified under the assumption of geometric similarity and operation in fully turbulent conditions (Li et al., 2006; Xing et al., 2009). Nevertheless, due to variability on impeller geometries, the impeller speed is not always an appropriate parameter to demonstrate the shear levels generated. Even though the impeller diameter is considered for the estimation of $u_{\text{tip}}$, $P/V$ is a more suitable measurement as it is a function of rotational speed, fluid properties, impeller sizing and working volume (Ghotli et al., 2013). It has been discussed that keeping $P/V$ constant in scale-up might result in a rise in the shear levels in the interior of the reactor, though together with $k_L a$ they represent the most common scale-up criteria for mammalian cell cultures (Platas Barradas et al., 2012; Dorceus, 2018; Tregidgo, 2021; Karimi Alavijeh et al., 2022).

Scaling among bioreactors with different volumes and geometries can be challenging due to the differences in flow dynamics involved that may impact reactor performance and process efficiency if not thoroughly examined (i.e. lead to poor mixing and/or high shear environments) (Li et al., 2006). Some research works have focused on the characterisation of the power requirements in small scale bioreactors. For example, Wyrobnik et al. (2021) measured the power requirements in the commercial 1 L Univessel® (Sartorius, un baffled) and compared the $P_0$ among marine, a 3BS and a novel impeller configuration (Wyrobnik et al., 2021). van Eikenhorst et al. (2014) examined the power characteristics in Single Use Bioreactors (SUBs) including the 3 L Mobius CellReady (Millipore, un baffled) and the 2.5 L Univessel® (Sartorius, un baffled) by using various axial impellers, and the results were similar to the corresponding glass vessels after direct comparison of $P_0$ (van Eikenhorst et al., 2014).
Focusing on mL-scales, Nienow et al. (2013) characterised the commercial ambr®15 (Sartorius), with rectangular shape, equipped with an elephant ear-like impeller and a sparger, and reported a $P_0 \approx 2.1$ based on CFD RANS simulations. They found that the $P_0$ was comparable to the one measured for a similar impeller (similar both in size ratios and type) in the cylindrical ambr®250 (250 mL) (Sartorius) bioreactor equipped with four baffles. Based on the combined findings for flow (presented in Section 1.2.4.2 and 1.2.4.4) and $P_0$ the authors suggested that the presence of a sparger in the 15 mL vessel might act equivalently to a baffle. In the same work, power number was found independent of $Re$ in the range $2,000 < Re < 10,000$ implying that the flow in this 15 mL vessel can be considered turbulent even at the low $Re$ limit ($Re \approx 2,000$) (Nienow, Rielly, et al., 2013a). Similarly, Rotondi et al. (2021) measured the $P_0$ of various impellers in the ambr®250. The vessel was unbaffled but was equipped with two probes for pH and temperature monitoring and a sparger. In accordance with the aforementioned findings, in their work $P_0$ plateaued in the range $2,500 < Re < 12,500$ whereas for all the impeller configurations studied including hydrofoils, elephant ear and paddle, the resulting $P_0$ was similar to values reported for baffled vessels (Ibrahim and Nienow, 1995; Rotondi et al., 2021). In the same context Collignon et al. (2016) used RANS simulations to estimate the $P_0$ for axial and radial impellers in a 200 mL STR equipped with two probes and a sample line at high $Re$ and observed similarity of the results with baffled tanks equipped with similar impeller types arguing that the minibioreactor baffles might be the probes (Collignon et al., 2016). These results indicate that the dimensions of bioreactor internals including probes and/or baffles relative to the small reactor size in the SDMs, impact the resulting $P_0$ in a similar way and will consequently influence the resulting $P/V$ which is one of the principal scaling parameters used in bioprocessing.

In the context of scaling-up/down, most scaling rules and design criteria have been adjusted and tested in fully turbulent conditions in large scale systems. However, alterations in flow regime and/or number and configuration of reactor internals in scale-down devices add extra challenges in hydrodynamics characterisation that should be addressed for process and scaling optimisation (Nienow, Rielly, et al., 2013b; Sandner et al., 2019).

1.2.5.1 The contribution of drag on Power consumption

While power consumption in STRs has traditionally been measured based on the torque of the impeller shaft, this method does not give insight with respect to the distribution of pressure forces generated by the impeller blades and reactor internals (i.e. probes and/or baffles) (Tay and Tatterson, 1985; Steiros et al., 2017b). Apart from eq. 2.2 the power can be
also determined based on the total drag force, $F_D$, as detailed in Section 2.3.5 in eq. 2.5-2.9, which can be decomposed to form (or pressure) and friction (or skin) drag (Tay and Tatterson, 1985). In principle both forms of drag are generated due to viscosity but are related to different phenomena: form drag is dominant for separated flow and is proportional to the cross-sectional area of the body whereas friction drag prevails for attached flows (i.e. no separation has occurred) and is analogous to the surface area exposed.

When the drag of an object is controlled by viscous forces, the object is called streamlined, whereas when pressure drag is higher, the body is called bluff. Whichever form of drag is the prevailing one is determined from the shape of the object (cf. Fig. 1.2). The most important parameter in the magnitude of form drag is the boundary layer separation resulting in a low pressure wake region formed behind the bluff bodies, as indicated in Fig. 1.2(a) and Fig. 1.2(b). Cylinders, spheres and flat plates are considered bluff bodies because at high $Re$, high and low surface pressure regions are formed in the front and at the back of the objects respectively and consequently, pressure (or form) drag is generated from the eddy motions produced due to flow separation caused by the fluid passage across the body (Houghton et al., 2017a). For the reduction of the excessive form drag, streamlining is of major importance as by increasing the surface area of the object, a shift of boundary layer separation occurs and as a result the wake and form drag are decreased (cf. Fig. 1.2(c)) (Houghton et al., 2017b). In Fig. 1.2 the aforementioned flow patterns are summarised, ranked in descending order of the generated form drag. In Fig. 1.2(a) the flow distribution around a flat plate is presented which can be indicative of the impeller blade. In Fig. 1.2(b) the flow around a sphere or cylinder is illustrated which is similar to the flow around a cylindrical probe or baffle. Finally in Fig. 1.2(c) a streamlined body is shown which can be used to reduce the generated form drag.

When a uniform flow is approaching a bluff body, for instance a cylindrical probe in a STR, flow separation occurs which can lead to either vortex detachment or vortex shedding. The Strouhal number ($St$) is associated with flow oscillations caused by convective acceleration of the flow field and can be used for the determination of the frequency of vortex oscillation. The $St$ number depends on the inertial and viscous forces dominating the flow and therefore it can be expressed as a function of $Re$. At high $Re$ ($Re>10^3$), the $St$$\sim$0.2-0.3 and leads to vortex shedding while at lower $Re$ ($Re<200$), $St$ is low and leads to vortex detachment (Katopodes, 2019). In STRs the flow generated by the impeller is usually turbulent thus based on empirical correlations $0.2 < St < 0.3$ and therefore, if a bluff body is encountered, such as a probe, two
symmetrical vortices will be formed around it (aka Karman vortices) (Kiya, Tamura and Arie, 1980; Katopodes, 2019)

The estimation of power requirements in STRs is limited to traditional methodologies based on torque measurements as the experimental determination of pressure distribution can be challenging (Steiros et al., 2017b). Tay et al. (1985) experimentally measured the pressure drop occurring across the blade of a 45° PBT to study the contributions of form and friction drag to the power number of that impeller. They reported a \( P_0 \sim 1 \) which compared well with values of \( \sim 0.9 \) reported in the literature for similar types of turbines. Moreover, in the same work, it was discussed that the dominant drag contribution was originated by the pressure (i.e. form drag), which is expected at high \( Re \) according to the literature, and was estimated to be two orders of magnitude higher than the friction drag (Tay and Tatterson, 1985). More recently, in the context of estimating STR power requirements and subsequently \( \varepsilon \) levels, Steiros et al. (2017) suggested a method to measure the instantaneous pressure distribution inside a STR through a pressure sensor and calculated the \( P_0 \) of a four-blade FBT and two novel fractal impellers, based on the form drag, from the integration of the measured pressure. After comparing the results with the \( P_0 \) estimated from torque measurements, they reported good agreement of the data between the two methodologies with an error of \( \sim 1.6\% \) for the FBT (Steiros et al., 2017b). Considering the limitations and challenges associated with the experimental determination of pressure distributions in stirred vessels, CFD can be used as an alternative to extract instantaneous pressure data for similar applications. Moreover, the aforementioned methodology for power estimation based on drag could be further expanded to predict the drag imposed by probes and/or baffles and suggest potential ways of minimising their contribution.

1.3 Summary of the literature survey

Continuous biomanufacturing and consequently perfusion cultures are continuously gaining increasing interest in the biopharmaceutical industry (Croughan, Konstantinov and Cooney, 2015). In this respect the development of scale-down devices, capable of multivariate process optimisation of high cell density perfusion cultures and the demonstration of the equivalence of the CQAs at different scales, is essential. Small scale (mL-scale) bioreactors have been extensively adopted within the biopharmaceutical industry not only as screening tools but also SDMs, some capable to operate under GMP and therefore useful to inform the late phases of clinical development (Sandner et al., 2019). The use of bioreactors in perfusion mode and
at high cell densities results in an increase in the metabolic demands of cell culture and highlights the need to maintain adequate oxygen transfer, nutrient homogeneity and low shear levels. The thorough understanding of fluid flow in stirred vessels is particularly important and can assist in the evaluation of the impact of critical design parameters on flow patterns underpinning upstream operations, such as mixing efficiency and gas dispersion (Karst et al., 2017).

Over the past years several studies have focused on the assessment of fluid dynamics within standardised vessel configurations (volumes of 10-70 L or even more) both experimentally and computationally. Previous experimental works focused on the characterisation of vortical structures and characteristic velocities for common impeller configurations for radial (Riet and Smith, 1975; Yianneskis, Popiolek and Whitelaw, 1987; Stoots and Calabrese, 1995; Escudié and Liné, 2003; Mochizuki et al., 2007; Steiros et al., 2017a) and axial (Kresta and Wood, 1993; Escudié, Bouyer and Liné, 2004) flow and described the dependency of flow patterns on design parameters including impeller diameter and clearance (Yianneskis, Popiolek and Whitelaw, 1987; Kresta and Wood, 1993). The impact of trailing vortices on mixing performance and turbulence has been mentioned by Van’t Riet, Bruijn and Smith, (1976) while Bouremel, Yianneskis and Ducci, (2009a) reported that the stretching and compression forces occurring in close proximity to the vortex core can contribute in improving gas and solid dispersions.

In the same context, the hydrodynamic environment has been thoroughly characterised via CFD through different modelling approaches. Strengths and weaknesses of RANS and LES models have been discussed for standardised vessels operating at high Re (Bakker and den Akker, 1994; Aubin, Fletcher and Xuereb, 2004a; M Coroneo et al., 2011), after comparing the results with comparable experimental data (Hartmann, Derksen and van den Akker, 2004; Delafosse et al., 2008). Assessment of turbulent characteristics has been performed both computationally and experimentally aiming at a better understanding of their behaviour across all flow characteristic scales. Previous experimental studies have focused on the characterisation of energy transfer among the main flow motions (i.e. mean, periodic and turbulent) (Escudié and Liné, 2003), direct determination of $\varepsilon$ (Ducci and Yianneskis, 2005a) and indirect estimation of $\varepsilon$ via applying flow decomposition techniques (Liné, 2016) while others underlined the assumptions taken in CFD modelling with respect to the resolution of turbulence (Delafosse et al., 2008, 2009). In this context a few studies have focused on computational characterisation of the flow at small scale via RANS and/or LES aiming at
analysing the flow patterns at the low Reynolds numbers that SDMs tend to operate ($Re \sim 2,000 \text{ - } 3,500$) and reported satisfactory agreement between mL and pilot scale with respect to the average velocity field and main flow patterns (Nienow, Rielly, et al., 2013a; Collignon et al., 2016; Rotondi et al., 2021).

Considering that baffles in STRs contribute to the improvement of mixing performance, the impact of baffle presence (Fan et al., 2021) and geometry (Atibeni and Gao, 2013) on the flow patterns has been assessed. For smaller scale systems, previous works reported the flow similarity between mL scale (15-250 mL) equipped with probes or other internals (i.e. sample lines, spargers) and larger fully baffled tanks, suggesting that the presence of probes in SDMs might provide the flow with enough baffling and give all the advantages that are related to a baffled configuration (Nienow, Rielly, et al., 2013a; Collignon et al., 2016; Rotondi et al., 2021).

Reactor geometry, flow regime and impeller type are key parameters affecting the development of macroinstabilities (MIs) in STRs. Roussinova et al. have thoroughly characterised MIs arising from PBTs and described the parameters that can trigger them (Roussinova, Grgic and Kresta, 2000; Roussinova, Kresta and Weetman, 2003, 2004). After examining various different impellers it was reported that the baffle number can trigger the presence of MIs with more pronounced results coming from radial impellers. The existence of MIs has been assessed by several methods including decomposition techniques (Ducci, Doulgerakis and Yianneskis, 2008; Doulgerakis, Yianneskis and Ducci, 2011) and Fast Fourier Transform (FFT) (Roussinova, Grgic and Kresta, 2000; Galletti et al., 2004; Galletti and Brunazzi, 2008) while their impact on turbulence and mixing has also been discussed (Roussinova, Grgic and Kresta, 2000; Ducci, Doulgerakis and Yianneskis, 2008; Doulgerakis, Yianneskis and Ducci, 2009).

Finally engineering parameters for bioreactor scale-up/down have been thoroughly reviewed for the application of animal cell culture (Xing et al., 2009; Platas Barradas et al., 2012). Power per unit volume ($P/V$) is one of the most applied criteria for reactor scaling and has been thoroughly investigated in the past for different impeller and vessel geometries (Bates, Fondy and Coprstein, 1963; Nienow and Miles, 1971; Karimi Alavijeh et al., 2022). More recently Nienow et al. (2013) and Rotondi et al. (2021) have discussed power requirements in the commercial SDMs ambr®15 and ambr®250(Sartorius) with two different volumes (15 mL and 250 mL) underlining potential limitations across small and larger scales which can lead to dissimilarity of flow regime and shear distribution and have important implications on
scaling parameters including oxygen transfer and power per unit volume (Nienow, Rielly, et al., 2013a; Xu et al., 2017; Rotondi et al., 2021).

This literature review highlights the current contributions in the engineering characterisation (experimental and computational) of standardised STRs and details research findings regarding the flow patterns, trailing vortices, MI and power consumption describing how these are impacted by the reactor design parameters including impeller size, clearance and baffle presence. With the exception of very few studies focused on the investigation of the engineering characteristics in small scale reactors (Nienow, Rielly, et al., 2013a; Collignon et al., 2016; Xu et al., 2017; Rotondi et al., 2021), the increasing interest towards the development of validated and qualified SDMs stresses the need to expand the existing knowledge on STR hydrodynamics in smaller scales where the probes and baffle dimensions relative to the reactor size can greatly affect the overall flow field and reactor performance.

1.4 The present contribution

The need for process efficiency and successful scale-up have rendered the characterisation of ml-scale STRs critical to describe mixing and turbulence and identify potential bottlenecks associated with process design. This comes in conjunction with the limited availability of small scale bioreactors that are tested and optimised to effectively operate in perfusion mode, where optimal mixing is essential for the successful performance of high cell density cultures.

Scale-down bioreactors are usually designed with a prior objective to provide optimal mixing performance which often affects the conservation of geometric similarity between small and larger scales. Due to fabrication and operating limitations, these dissimilarities are more evident as the reactor scale becomes smaller and might result in significant implications on the process performance achieved. Moreover, the matching of (bio)chemical processes in small and larger scales often results in altering critical operating parameters in the former (e.g. reduction in rotational speed), potentially affecting the flow regime among scales and mean flow characteristics and therefore the dynamic similarity across the different scales (Nienow, Rielly, et al., 2013b; Collignon et al., 2016).

Taking under consideration the aforementioned scaling issues between small and large scale upstream processes, a systematic characterisation of engineering features in scale-down reactors is particularly important for the development of reliable SDMs and has rarely been reported. To date studies on small scale reactors has been limited to spatial distributions of ensemble-averaged flow quantities (i.e. mean velocity and turbulence) obtained either
computationally or experimentally and estimation of power number with or without the presence of internals (Nienow, Rielly, et al., 2013a; Collignon et al., 2016).

The aim of this work is to fully assess the flow patterns developed in a small scale reactor, designed by Tregidgo et al. (2021) and optimised in continuous mode of operation for antibody producing CHO cell cultures (Tregidgo, 2021). For this reason, the vessel hydrodynamics at the typical culture conditions corresponding to intermediate Re (Re=3,732) are thoroughly characterised via CFD modelling, including RANS and LES approaches, and validated via particle image velocimetry (PIV) experiments. Thereinafter, the impact of design parameters is assessed with a particular focus on baffle number and size, in order to evaluate the dependency of reactor hydrodynamics on key geometrical features. In this context, three configurations of the aforementioned scale-down reactor have been tested, one unbaffled (UB) and two baffled configurations with baffles of increasing size. Reactor hydrodynamics are assessed across the different geometries with respect to time, phase resolved velocities and turbulence levels. Moreover, the structure and evolution of trailing vortices are assessed based on several visualisation methodologies. The stability of impeller trailing vortices is quantitatively examined via flow decomposition techniques and correlated to highly energetic jet instabilities the intensity of which increases with baffle size and absence of impeller disk.

The power requirements of the various vessel configurations are examined and compared with data from commercial SDMs with working volumes 60 mL – 1 L and with the available literature in order to obtain an end-to-end understanding of the design parameters affecting them. The analysis is expanded by characterising the impact of the presence and size of internals (baffles and/or probes) on the resulting $P_0$, and consequently $P/V$, in order to elucidate how these affect established scaling criteria. In addition, based on previous considerations that probes can act in a way equivalent to baffles (Nienow, Rielly, et al., 2013a; Rotondi et al., 2021), the flow dynamics in the presence of both probes and baffles are studied via LES in order to observe differences in power consumption in the presence of both. Extracted pressure and velocity data are ultimately used to estimate drag and optimise probe geometry contributing to the reduction of overall power requirements.

The present work contains new information on the investigation of reactor flow field, trailing vortex dynamics and scale-up. To the best of the author’s knowledge, it is the first time a detailed validated hydrodynamic analysis is reported in a SDM optimised to operate in perfusion mode and critical design parameters, such as baffle and probe presence and size,
are evaluated with regards to their effect on the flow dynamics and power consumption. Flow sensitivity on reactor internals, indicates the importance of parameter optimisation when small scale STRs are tested and highlights the significance of detailed understanding of the hydrodynamics for optimising reactor design features.

1.5 Outline of the thesis

The remainder of this thesis consists of five chapters. In chapter 2, the main bioreactor configurations investigated in the current work are presented, along with the experimental and computational methodologies implemented for the characterisation of reactor hydrodynamics and power characteristics. Moreover, the parameters applied for the implementation of CFD are detailed along with the characteristics of model tuning including mesh independence studies, calculations of turbulent spatial and time scales for mesh refinement and initial model screening for successful setup of LES. The processing techniques used for the analysis of the resulting flow data are also described including post-processing methods for experimental and computational data, tools for frequency analysis and the basic principles of POD technique.

Chapter 3 presents the results obtained on bioreactor hydrodynamics for three different vessel configurations. In this chapter the validation of CFD via PIV is illustrated for the entire range of STRs used, both in terms of velocity and turbulence, and performance comparison between different CFD models are discussed.

The impact of the presence of baffles and baffle size on the formation and stability of impeller trailing vortices is described in chapter 4. The trailing vortices are characterised based on the instantaneous and phase resolved velocity vector fields and vorticity magnitude. Fluctuations of the angle of the impeller jet are investigated to assess emerging jet instabilities while POD and FFT are used to extract dominant spatial modes and corresponding frequencies affecting the underlying flow patterns. The influence of baffle presence and size on those instabilities is discussed and correlated with the absence of the impeller disk.

In chapter 5 the impact of internals including baffles and/or probes on power consumption is examined and compared for two commercial small scale bioreactors. Moreover, changes in bioreactor hydrodynamics are examined in the presence of probes and baffles focusing on the estimation of form (or pressure) drag based on CFD LES data. Finally, novel probe configurations are suggested for the reduction of power consumption.
In chapter 6, a summary of the main conclusions extracted from this project is outlined along with recommendations for future work.
Figure 1.1: Illustration of vortex formation mechanisms for (a) a standard Rushton Turbine (RT) described by Riet and Smith, (1975) and (b) a Flat Blade Turbine (FBT) described by Winardi and Nagase, (1994).
Figure 1.2: Flow patterns over (a) a flat plate, (b) a sphere or cylinder and (c) a streamlined body (here symmetric airfoil) ranked based on descending order of the generated form drag. On the right hand side, the relative amount of the generated drag is illustrated for each condition (Heisler, 2002).
Chapter 2: Bioreactor Configurations, Experimental and Computational Techniques

2.1 Introduction

In this chapter the bioreactor configurations and experimental and computational techniques used in the current work are presented. STRs size, geometry, impeller and internal types are described in detail in Section 2.2. Experimental setup and methodology of power consumption measurements are presented in Section 2.3. Section 2.4 provides an outline of the characteristic parameters of the computational approaches implemented for the assessment of flow dynamics and details the principles of operation of Particle Image Velocimetry (PIV) used for experimental validation of CFD models. Processing methods of reactor hydrodynamics, including assessment of velocity and turbulence, and the Proper Orthogonal Decomposition (POD) technique applied for the analysis of flow instabilities are outlined in Section 2.4.

2.2 Bioreactor configurations

For engineering characterisation, a small scale STR with a working volume of 250 mL has been used and is presented in Fig. 2.1. The bioreactor is a custom made SDM, designed by Tregidgo et al. (2021), successfully used in antibody producing CHO cell cultures in perfusion mode (Tregidgo, 2021). The vessel has a flat bottom with internal diameter $D_T = 67$ mm and is filled up to $H_L = 1.05D_T$. In Fig. 2.1(b)-Fig. 2.1(d), the bioreactor configurations in which analysis of hydrodynamics has been conducted, are illustrated alongside sizing characteristics of the impellers and internals (i.e. baffles and probes) used. For the purposes of hydrodynamic analysis two types of radial impellers were studied: a Flat Blade Turbine (FBT) with six blades and a standard Rushton Turbine (RT). Both impellers were of equal diameter, blade thickness and blade width ($D_i \sim 0.44D_T$, $t_{blade} = 0.05D_i$ and $w_b = 0.26D_i$, respectively) and were mounted on a 4 mm diameter shaft and rotated at 250 rpm corresponding to $Re = ND_i^2v^{-1} = 3,732$. The impeller shaft is driven by an N-series Allen Bradley Motor unit connected to an Ultra 3000
drive and controlled using the Ultraware software (Rockwell Automation, Milwaukee, WI, USA), to accurately set the desired rotational speed, \( N \). All experiments were conducted in a cylindrical transparent vessel immersed in a rectangular trough made from acrylic, which minimised optical distortions caused by the curved surface of the cylindrical tank (Fig. 2.1(a)). Detailed information about the vessel and impeller geometries and sizing parameters is presented in Fig. 2.1 and in the first row of Tables 2.1-2.2, while a summary of investigation conducted for each configuration is described in Table 2.3.

The impact of internal presence, size and geometry on power consumption has been examined across different vessels. For the 250 mL acrylic mimic (illustrated in Fig. 2.1) seven different configurations have been used including an unbaffled-no internal (UB-NI) configuration, two baffled configurations with B1 and B2 (B1<B2), two configurations with probes of different diameters, \( D_{Pa} = 0.12D_T \) and \( D_{Pb} = 0.18D_T \), and equal lengths \( L_{Pa} = L_{Pb} = 0.9H_L \), and finally two configurations combining B1 with each of the probe types \( Pa (Pa+B1) \) or \( Pb (Pb+B1) (Pa<Pb) \). The sizes of those probes corresponded to volume blockage (V(\%)) (i.e. probe volume over the total working volume) of 2.8\% and 6.3\% for the small (\( Pa \)) and big (\( Pb \)) probes respectively. The geometry and size of the probes were similar to those used by Tregidgo et al. (2021) (Tregidgo, 2021) in real culture conditions for pH and oxygen monitoring and control. Sizing parameters of the probes used in the custom made 250 mL small scale vessel are shown in Fig. 2.1(d) and in the first row of the Tables 2.1-2.2.

The power number data obtained for the 250 mL vessel prototype (Fig. 2.1) were compared with power number measurements across bioreactors with different impellers, working volumes and internal geometries. For this purpose, two additional SDMs were used: the 60 mL EasyMax\textsuperscript{TM} (Mettler-Toledo) and an acrylic mimic of the commercially available 1 L Univessel\textsuperscript{®} (Sartorius) Glass bioreactor presented in Fig. 2.2 and Fig. 2.3 respectively. For the 60 mL EasyMax\textsuperscript{TM} (Mettler-Toledo), different types of internals were used, including baffles, addition lines and probes with V(\%)~0-8.4; sizing parameters and vessel configuration are presented in Fig. 2.2 and in the second row of Tables 2.1-2.2. For the 1 L Univessel\textsuperscript{®} (Sartorius) power characteristics were examined in the presence of radial (six blade FBT) and axial (45° 3-Blade Segment, 3BS) impellers and different internal types: culture internals (i.e. present in the reactor in real culture conditions) with V(\%)~0-3 – when the 45° 3BS was used – and novel probes V(\%)~0.2-1 when the FBT was used. The novel probes, had a streamlined shape with varying chord lengths (\( l \)) and thicknesses (\( \delta \)), illustrated in Fig. 2.3. Vessel and internal sizing characteristics for the 1 L Univessel\textsuperscript{®} (Sartorius) are presented in the third row of Tables 2.1-
2.2. $P_0$ data for this analysis including the 60 mL EasyMax™ (Mettler-Toledo) and the axially-45° 3BS agitated 1 L Univessel® (Sartorius) were collected by two other researchers in this research group, Dr Anne DeLamotte and Mr Tom Wyrobnik, and were analysed and interpreted in the present study. $P_0$ data for the radially agitated 1 L Univessel® (Sartorius) were collected by Mr Eugene Ting. All bioreactor configurations used in the current work alongside the analysis implemented for each vessel, are presented in Table 2.3.

For computational and experimental fluid dynamics investigation, the working fluid was water with density $\rho_L=1,000$ kg/m$^3$ and dynamic viscosity $\mu=0.001003$ kg/ms. All experiments and simulations were conducted at room temperature. The rotational speed was 250 rpm and corresponded to a Reynolds number $Re=3,732$. Fluid dynamics investigation was carried out, both computationally and experimentally, at $N=250$ rpm ($Re\sim3,732$) in order to mimic the operating conditions of the high-cell density biological experiments conducted by Tregidgo (2021). As described in Tregidgo (2021) the majority of cell culture experiments for all three bioreactor modes of operation, including batch, fed-batch and perfusion, were performed at a rotational speed of 250 rpm, therefore the flow analysis at $Re=3,732$ aimed to thoroughly investigate the flow patterns corresponding to those parameters.

For power number measurements the working fluid was a water-glycerol mixture with density $998.15$ kg/m$^3<\rho_L<1,260$ kg/m$^3$ and viscosity $9.9 \cdot 10^{-4}$ m$^2$/s<$\nu<1.02 \cdot 10^{-6}$ m$^2$/s with higher and lower values corresponding to 100% v/v glycerol and water concentration respectively. $Re$ varied in the range $1<Re<15,000$ in order for power consumption data to be acquired in laminar, transitional and turbulent flow regime.

2.3 Power consumption
2.3.1 Principles of operation
The power input, $P$ [W], of a system can be calculated based on the rotational speed, $N$ and impeller torque, $M$[Nm], and based on that the impeller power number $P_0$ can be estimated. There are many available methods for the measurement of reaction torque including the use of dynamometers based on pneumatic bearings which can be accurate for bench scale STRs (Kaiser et al., 2017; AU - Kaiser et al., 2018).

The principle of operation for these devices is based on Newton’s third law. The agitator exerts a mechanical force on the working fluid to which the latter presents resistance. Under standard operating conditions the fluid generates a torque on the impeller that is transmitted
to the motor through the shaft. In order to quantify the torque, a support system can be used allowing the vessel to rotate without friction. In this way the total torque can be measured using a force sensor, suitably calibrated and mounted via a mechanical coupling.

### 2.3.2 Experimental setup

The experimental setup for power input measurements is schematically shown in Fig. 2.4. The cylindrical air bearing made in brass had an external diameter of 100 mm, a total height of 53.5 mm (Biochemical Engineering Workshop, UK) and consisted of three parts: a main body, an air distribution plate and a freely rotating stage. Air pressurised at >0.5 bar is injected into the main body via a nozzle through a silicon tubing and reaches the distribution plate generating a uniform layer of air that lifts the rotating stage and ensures smooth motion of the bearing. For the execution of the measurements the bioreactors were placed on a vessel holder that was screwed on top of the rotating stage. Hence upon impeller agitation the vessel holder and stage rotated with the same speed. The force required to stop the vessel rotation was measured using a digital force gauge (DFG55-10, Omega Engineering, Manchester UK), with a sensitivity range 2-500 N. The lever arm (i.e. the distance from the point of measurement to the tank axis), \( l_r \), was 45 mm and 52.5 mm for the 250 mL custom made bioreactor (Fig. 2.1) and 1 L acrylic mimic of Univessel® Sartorius (Fig. 2.3), respectively. The measurements (10 measurements acquired per second), were averaged over a 60 second period and run in triplicates. Before each measurement the force gauge was tared and the mixing fluid was allowed to reach steady state for 60 s.

Measurements were conducted at room temperature (21°C±0.5). The working fluids were water or mixtures of water and glycerol (99% Fisher Scientific, UK). The addition of glycerol allowed the determination of the power number at lower \( Re \):

\[
Re = \frac{ND_i^2 \rho_L}{\mu} = \frac{ND_i^2}{\nu} \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.1)
\]

where \( \mu \) and \( \nu \) are the dynamic and kinematic viscosities of the working fluid and \( \rho_L \) is the fluid density. The temperature of the working fluid was regularly checked during the measurements. All configurations were agitated by a broad speed range 50 rpm<\( N <800 \) rpm which was dependent on the system’s inertia and force gauge sensitivity.
2.3.3 Data processing methodology

The torque, \( M [Nm] \), produced by the impeller was calculated as the product of the lever arm and the force exerted by the rotating tank on the force gauge. Power input \( (P) \) can be computed directly from the impeller rotating speed and the measured force according to (eq. 2.2):

\[
P = 2 \pi N F l_r = 2 \pi NM \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.2)
\]

where \( l_r, F \) and \( N \) are the lever arm, the measured force and the rotational speed, respectively. The dimensionless \( P_0 \) can be then calculated as follows (eq. 2.3):

\[
P_0 = \frac{P}{\rho \nu N^3 D_i^5} \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.3)
\]

The measured power is directly proportional to the overall energy dissipation rate which can be estimated based on eq. 2.4.

\[
\epsilon_v = \frac{P}{\rho \nu V} \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.4)
\]

2.3.4 Sources of error

Several sources of error should be considered when carrying out power number measurements. Firstly, torque measurements should be conducted with caution to ensure that torque losses due to friction applied by bearings, shaft seals and motor are minimised. Moreover, the accurate operation of the force sensor can be listed as an additional source of error. The technique described in Section 2.3.2, which was used in the present study, can only give acceptable results when operational conditions cover the sensitivity range of the force gauge used and is consequently dependent on the rotational speed and the reactor scale.

The properties of the working fluid form an additional source of error. The temperature of the working fluid should be carefully monitored especially at high glycerol concentrations due to its high sensitivity to temperature changes during agitation. In the present work, during the torque measurements, the temperature of the working fluid was measured via a thermocouple before and after each measurement. Average temperature values were used for the estimation of fluid properties \((\rho, \mu)\) and \(Re\).

Finally an important source of error could come from the level of the table where the air bearing is placed. In this work the levelling of the table was conducted with caution but was manual, and this could have introduced some bias on the measurements at a certain direction of rotation. To ensure the reactor rig and experimental platform were balanced, a spirit level
was used throughout the experimental process. To minimise this systematic error the final torque value was obtained as an average of the torque measured in both directions of rotation (clockwise (CW), counter-clockwise (CCW)), considering that the impeller used in the current work was a FBT (pitch of the blade 90°) and therefore expected to generate equivalent flows in both directions of rotation. An ideal solution to avoid this source of error would be to use a self-levelling table.

2.3.5 Power calculation based on drag

Apart from the conduction of experimental measurements (Section 2.3.1-2.3.4), power consumption was also computed based on the total drag force \( F_D \) according to eq. (2.5-2.6).

\[
P = F_D U_\infty \quad \ldots 
\]
\[
F_D = \frac{1}{2} C_D A \rho L U_\infty^2 \quad \ldots 
\]

where, \( U_\infty \) is the fluid free stream velocity (i.e. the velocity of the stream approaching) and it is related to the impeller tip velocity. The drag force is usually expressed as a function of the drag coefficient, \( C_D \), the projected area, \( A \), the density of the fluid, \( \rho_L \), and the free stream velocity, \( U_\infty \) (eq. 2.6) (Tay and Tatterson, 1985).

The total drag force can be calculated by the sum of form (or pressure) and friction (or skin) drag contributions which are based on the integration of the total pressure and shear stress respectively as indicated in eq. 2.7-2.9:

\[
F_D = F_{form} + F_{friction} \quad \ldots 
\]
\[
F_{form} = \int p dA \quad \ldots 
\]
\[
F_{friction} = \int \tau dA \quad \ldots 
\]

In eq. 2.8-2.9 \( p \) and \( \tau \) are the total pressure and shear stress respectively. For the drag coefficient, \( C_D \), in eq. 2.6 both form and friction contributions in drag are considered (Tay and Tatterson, 1985).
2.4 Flow analysis and processing methods

2.4.1 Particle Image Velocimetry

2.4.1.1 Principles of operation
Particle Image Velocimetry consists of a laser light sheet which is used to continuously illuminate the working fluid seeded with tracer particles. These should perfectly follow the fluid flow and scatter the laser light. The tracer particles are used together with a cut-off filter that lets through the wavelength emitted by the particles once they are excited and at the same time blocks any background light thus improving the visualisation of particle movement. Double exposure frames are then recorded by a high-speed camera, with a certain time step, \( dt \), and processed by a software for the estimation of particle displacement from which fluid velocity is then derived. During post-processing the collected images are divided into interrogation areas and particle displacement is estimated based on a cross-correlation algorithm. Interrogation areas should include 5-10 tracer particles (Escudié and Liné, 2003) under the assumption that all of them move towards the same direction between the different frames.

2.4.1.2 Experimental setup
A sketch of the PIV setup and a picture of the actual configuration used are shown in Fig. 2.5. 2D PIV time and phase resolved measurements of the instantaneous radial and vertical velocity components, \( u_r(\varphi, t) \) and \( u_z(\varphi, t) \), were obtained for two different vertical planes and the instantaneous radial and tangential velocity components, \( u_r(\varphi, t) \) and \( u_\theta(\varphi, t) \) were obtained for five horizontal planes (Fig. 2.5 (c)). The vertical planes were located at different azimuthal distances from the baffles: the cross-section denoted as P₁ was 30° after the baffle, and cross-section P₂ half way between two consecutive baffles (Fig. 2.1 and Fig. 2.5(c)). The horizontal planes were located at different tank elevations \( 0.15 < z/D_T < 0.56 \) (Fig. 2.5(d)). The plane of measurement was illuminated by a laser of thickness of approximately 1 mm in the measurement region and was obtained from a green Diode-Pumped Solid State (DPSS) Laser with an output power of 500 mW. A 45° mirror was placed underneath the rig to redirect the laser vertically (Fig. 2.5). The working fluid was milli-Q water seeded with Rhodamine-coated Polymethyl methacrylate particles with an average diameter \( d_p = 20-50 \mu \text{m} \) and density \( \rho_p = 1.19 \text{ g/cm}^3 \) (Dantec Dynamics A/S). The particle Stokes number ranged \( \tau_p \sim 6-60 \mu\text{s} \) (eq. 2.10). The Stokes number was several orders of magnitude below the average and maximum Kolmogorov timescale, \( \tau_{K,\bar{\varepsilon}} = 76,000 \mu\text{s} \) and \( \tau_{K,\bar{\varepsilon}\text{max}} = 900 \mu\text{s} \), which were estimated based on
average $\bar{\varepsilon}$, and maximum $\varepsilon_{max}$ values of energy dissipation rate, obtained from CFD simulations (eq. 2.11). The 1MP intensified camera (Dantec Dynamics A/S), was equipped with a 45 mm lens and mounted an orange light cut-off filter with a wavelength of 570 nm, which allowed maximisation of seeding particles detection and minimisation of laser reflection at the vessel and impeller surfaces.

$$\tau_p = \frac{\rho_p d_p^2}{18\mu}$$  \hspace{1cm} (2.10)

$$\tau_K = \left(\frac{v_L}{\varepsilon}\right)^{0.5}$$  \hspace{1cm} (2.11)

Image acquisition was conducted in a double frame mode. The frequency of frame acquisition was optimised in order to ensure minimal losses of particles from the plane of measurement due to increased tangential motion (acquisition frequency has to ensure that minimal amount of seeding particles move out of plane). Depending on the vessel configuration (baffled/UB) and the tank region investigated (impeller versus bulk tank regions) the frame rate varied from 2,000 to 4,000 Hz (time step size, $dt = 0.25 - 0.5 \text{ ms}$). The spatial resolution used for the experiments was 1.1 mm, which is consistent with the order of magnitude of the Taylor microscale ($\lambda \sim 0.5-1 \text{ mm}$) found in the literature (Escudié and Liné, 2003; Ducci and Yianneskis, 2005a) and estimated from CFD simulations for $Re=3,732$ as described below (see Sections 2.4.2). The experimental parameters used, including spatial, temporal resolution and vector count, are summarised in Table 2.4 for each bioreactor configuration. Erroneous vectors were detected by applying velocity limits (approximately $\pm 2.5 u_{tip} = 1 \text{ m/s}$) and discarding outliers with velocities five times greater than $u_{tip}$, that corresponded to $\sim 2\%$ of the generated vectors. Ensemble and phase averaged measurements were obtained from 19,200-38,360 instantaneous velocity fields corresponding to 40 impeller revolutions, depending on the frame rate selected ($0.5-0.25 \text{ ms}$), and 500 instantaneous velocity fields for each phase angle, respectively.

For phase resolved measurements the PIV system was coupled with the MPL Servo motor encoder, which signalled the impeller position at $\varphi=0^\circ$ with a pulse. The encoder is connected to a timing box in order for the camera to be synchronised with each impeller position under investigation. For the conduction of phase resolved measurements and the acquisition of data at different phase angle ($\varphi$), a time delay from zero position ($\varphi=0^\circ$) was set. For example, considering that $N=250 \text{ rpm}$, the impeller period is 0.24 s. Therefore, for $\varphi=30^\circ$ a time delay of 0.02 s was set at the timing box software and implemented from the encoder signal pulse.
2.4.1.3 Data processing methodology

PIV data was post-processed via PIVlab 2.34 (Thielicke, W. and Sonntag, 2021) using an adaptive correlation analysis, with initial and final interrogation areas of 128x128 and 32x32 pixels² for the baffled configuration with B1 (three-pass analysis) and 128x128 to 16x16 pixels² (four-pass analysis) for the UB configuration and baffled vessel with B2, respectively (Table 2.4). According to the adaptive correlation analysis an initial approximation of the local velocity is obtained in the largest interrogation area (128x128 pixels in this case) which is then used in multiple passes with higher resolution (i.e. smaller interrogation areas) until the final pass, which corresponds to the highest resolution (32x32 and 16x16 pixels² for B1 and UB/B2 configurations respectively). Between the passes, 50% overlap was used which resulted in a distance between adjacent vectors of 16 and 8 pixels, depending on the vessel configuration used, corresponding to a spatial resolution of 1.1 and 0.5 mm, respectively (Table 2.4). Velocity and turbulence data processing methods are described later in Section 2.4.3.

2.4.1.4 Sources of error

Several sources of errors should be taken under consideration during the conduction of PIV experiments. The accuracy of PIV measurements is dependent on various parameters including seeding concentration, acquisition frequency, area size of PIV measurement and spatial resolution. In principle, the maximisation of the size of the area of measurement is desired, in order to globally assess velocity distribution throughout the investigated plane. However, this is inversely proportional to the refinement of temporal and spatial resolution which must be low enough to accurately resolve the flow and predict fluid velocities. It is therefore important to ensure the temporal and spatial resolution are not compromised and are fine enough to capture the motion of the seeding particles especially in areas of higher velocity and turbulence.

The laser alignment is one additional source of error and uncertainty. The laser was manually aligned with the impeller blade at $\varphi=0^\circ$ at reduced intensity, through visual inspection. This process was repeated for each different bioreactor configuration investigated. To ensure the reactor rig and experimental platform were perfectly balanced, a spirit level was used throughout the experimental process. This type of error is considered to be systematic, while its impact on the measured data is assumed to be low.

Reflections coming from baffles and impeller blades can be listed as supplementary error sources. To minimise reflections, acrylic mimics of the stainless steel impeller and baffles were used. Furthermore, a source of uncertainty is the seeding density of the tracer particles.
Seeding density should be high enough to ensure that the number of particles is monitored for accurate velocity prediction after auto- and cross-correlation analysis. Seeding density was kept consistent throughout the experiments of the current work at \( \approx 1 \mu L/mL \).

Finally an important source of error in experimental techniques stems from ensuring that the sample size used for the experimental flow prediction is large enough to minimise statistical errors and establish reproducibility. For this reason, the mean velocity magnitude (calculated based on eq. 2.12) was estimated for increasing sample size for both phase and time resolved measurements at three different points of the flow around the impeller. The sample size corresponded to the number of frame pairs (i.e. revolutions considering that each pair of frames is collected per one impeller revolution) and the number of total frames for phase resolved and ensemble averaged measurements, respectively. The test for statistical convergence has been conducted for multiple phase angles, \( \varphi \), but is presented in Fig. 2.6 indicatively for \( \varphi = 30^\circ \). It is evident that when impeller revolutions exceed 100 and 5 (5 revolutions corresponded to 2,400 frames for a time step of 0.5 ms) for phase and time resolved measurements respectively, the average velocity magnitude of all the points considered tends to converge to a nearly constant value. Based on those results all the PIV measurements presented in the current work were conducted with a sample size \( N_S = 500 \) and 40 revolutions for phase and time resolved data respectively.

\[
\frac{|u|}{u_{tip}} = \sqrt{\frac{\Sigma_{i=1}^{N_i} (u_i^2(\varphi, t) + u_z^2(\varphi, t))}{N_S}} \quad \text{(2.12)}
\]

### 2.4.2 Computational Fluid Dynamics

#### 2.4.2.1 Simulation methodology and principles

The foundation of CFD simulations is based on the Navier-Stokes (N-S) equations. For STRs the incompressible N-S equations are (eq. 2.13-2.14).

\[
\nabla \cdot u = 0 \quad \text{.......................... (2.13)}
\]

\[
\frac{\partial u}{\partial t} = \nabla \cdot \left( -uu - \frac{p}{\rho_L} \delta + 2\nu S \right) \quad \text{.......................... (2.14)}
\]

where eq. 2.13 and 2.14 correspond to mass (continuity equation) and momentum conservation, respectively. The equations must be subjected to zero slip boundary condition on the tank wall and rotating impeller. In eq. 2.13 and 2.14, \( t, u, p, \rho_L \), and \( \nu \) represent time,
velocity, pressure, density and kinematic viscosity while $S$ is the strain rate tensor (Gillissen and den Akker, 2012). For the accurate prediction of the flow and turbulence eq. 2.13 and 2.14 should be explicitly solved throughout the total fluid volume via Direct Numerical Simulations (DNS) approach, however two alternative methods like Reynolds averaging and filtering are commonly used in order to reduce computational time and power (Gillissen and den Akker, 2012).

In Reynolds averaging, Reynolds Averaged Navier-Stokes (RANS) are used, which solely solve the mean flow field, and are combined with additional equations for modelling of turbulence kinetic energy ($k'$) and energy dissipation rate ($\epsilon$) to account for the effects of turbulent fluctuations. Besides their simplicity, the robustness of RANS models in STR turbulent flows has been discussed by many research groups (Montante et al., 2001; Aubin, Fletcher and Xuereb, 2004b; Delafosse et al., 2008; Joshi et al., 2011a; Singh, David F Fletcher and Nijdam, 2011) and there is general agreement on their satisfactory use as tools to reproduce the mean flow (Joshi et al., 2011a; Gillissen and den Akker, 2012).

In the filtering approach, part of the turbulent spectrum of the flow, which corresponds to the larger and less isotropic eddies, is directly resolved whereas eddies smaller than the grid spacing are modelled via a subgrid scale model. Models based on this approach include, among others, Large Eddy Simulations (LES), Detached Eddy Simulations (DES) and Scale Adaptive Simulations (SAS) (Roussinova, Kresta and Weetman, 2003; Menter and Egorov, 2005; Gillissen and den Akker, 2012; Gimbun et al., 2012).

Two approaches can be used to account for impeller rotation in CFD simulations in the context of STR operation: the Multiple Reference Frame (MRF) and the Sliding Mesh (SM) approach. MRF approach, is a steady state approximation in which the relative motion of the moving zone with respect to the adjacent zones is not taken into account, therefore the distance between the impeller blades and the vessel wall/baffles is considered to be sufficient to ensure the flow in the impeller region remains unaffected from the rest of the tank (thus assuming time independency in the impeller motion) (Aubin, Fletcher and Xuereb, 2004b; Alcamo et al., 2005; de Lamotte et al., 2018b). When the SM method is used the transient impeller-baffle interactions are captured along with the periodic unsteadiness caused by the impeller motion (Aubin, Fletcher and Xuereb, 2004b; de Lamotte et al., 2018b). Thus SM forms a more realistic approach when STR flows are simulated and has been used in CFD modelling of both baffled (Aubin, Fletcher and Xuereb, 2004b; Hartmann et al., 2004; Delafosse et al., 2015; Collignon et
al., 2016) and unbaffled reactors (Alcamo et al., 2005) as it considers the inherently transient nature of the flow.

In the current work, CFD simulations were conducted via both RANS and LES approaches while for the simulation of impeller rotation both techniques, MRF and SM, were implemented. RANS modelling was employed for mesh independence studies and estimation of average and maximum values of spatial and temporal length flow scales. Based on these, the spatial and temporal resolution for LES was determined. For CFD simulations the commercial software ANSYS (version 19.2) was used to solve the flow governing equations inside five scale-down STRs configurations mentioned in Section 2.2 and presented in Tables 2.1-2.3. A summary of the different conditions applied in the simulation processes is indicated in Table 2.8.

### 2.4.2.2 RANS modelling

When RANS simulations are used only the Reynolds averaged part of the flow is solved while the part related to turbulent fluctuations is modelled. After Reynolds averaging is applied to N-S equations, eq. 2.13 and 2.14 can be rewritten as:

\[ \nabla \cdot \bar{u} = 0 \]  
\[ \frac{\partial \bar{u}}{\partial t} = \nabla \cdot \left( -\bar{u}\bar{u} - \frac{\bar{p}}{\rho_L} \delta + 2\nu \bar{S} + R \right) \]  
\[ (2.15) \]
\[ (2.16) \]

where \( \bar{u}, \bar{p} \) and \( \bar{S} \) represent the Reynolds averaged velocity, pressure and strain rate tensor respectively. The equations 2.15 and 2.16 are similar to equations 2.13 and 2.14 despite the term \( R \), which appears due to averaging and represents the Reynolds stress. Reynolds stresses are modelled and related to the mean flow gradients via the Boussinesq hypothesis which is applied to all the single- and double-equation closure models (e.g. Spalart-Allmaras and k-ε models) and is associated with the simulation of turbulent viscosity.

\[ R = -\bar{u}\bar{u} + \bar{u}\bar{u} = -\bar{u}'\bar{u}' \]  
\[ (2.17) \]

In two-equation closure models (i.e. k-ε and k-ω), two additional transport equations are solved which correspond to turbulence kinetic energy and energy dissipation rate while turbulent viscosity (\( \mu_t \)) is obtained as a function of \( k' \) and \( \varepsilon \). Although two-equation closure models allow the estimation of turbulent time and length scales, they operate under the assumption of turbulence isotropy which may perform satisfactorily in cases where the flow is dominated by unidimensional shear stresses but may lead to turbulence damping in the cases of multidimensional turbulent flows. The Reynolds Stress Model (RSM) can be used as
an alternative in those types of flows in which each Reynolds stress component is explicitly
solved resulting in six equations for each one of the six components of the Reynolds stress
tensor, in combination with a complementary equation for the modelling of 𝜀 (7 equations in
In the current study screening simulations with different RANS models were carried out to
determine the model that could be most suitably used as LES precursor. The RANS models
used included k-ε Standard, k-ε RNG, k-ε Realizable and RSM and are briefly described below
in terms of main features and equations used for the prediction of 𝑘’ and 𝜀. The main
characteristics of the models are summarised in Table 2.5 alongside relevant literature.

2.4.2.2.1 k-ε Standard
Turbulent kinetic energy and energy dissipation rate are modelled based on the following
equations according to the k-ε Standard model:
∂
∂
∂
μt ∂k
(ρ𝐿 k′) +
(ρ𝐿 k′Ui ) =
[(μ + )
] + Gk + Gb − ρ𝐿 ε − YΜ + Sk … … … … . . (2.18)
∂t
∂xi
∂xj
σk ∂x𝑗
∂
∂
∂
μt ∂ε
ε
ε2
(ρ𝐿 ε) +
(ρ𝐿 εUi ) =
[(μ + ) ] + C1ε (Gk + C3ε Gb ) − C2ε ρ ′ + Sε … .. (2.19)
∂t
∂xi
∂x𝑗
σε ∂xj
k′
k
μt = ρ𝐿 Cμ

k′2
ε

… … … … … … … … … … … … … … … … … . (2.20)

In eq. 2.18, 𝐺𝑘 and 𝐺𝑏 indicate the production of 𝑘’ as a result of velocity gradients and
buoyancy respectively and 𝑌𝛭 represents the fluctuating dilatation and its contribution in
turbulence in compressible flows. 𝐶1𝜀 , 𝐶2𝜀𝜌 and 𝐶3𝜀 are constants, 𝜎𝑘 and 𝜎𝜀 are the Prandtl
numbers of 𝑘’ and 𝜀 respectively and 𝑆𝑘 and 𝑆𝜀 are source terms. The turbulent (eddy) viscosity
(𝜇𝑡 ) is described as a function of 𝑘’ and 𝜀 according to eq. 2.20. The model constants 𝐶1𝜀 , 𝐶2𝜀 ,
𝐶𝜇 , 𝜎𝜀 and 𝜎𝑘 have the following default values (ANSYS FLUENT Theory Guide Release 14.5,
2013; Koerich and Rosa, 2016):
𝐶1𝜀 =1.44, 𝐶2𝜀 =1.92, 𝐶𝜇 =0.09, 𝜎𝜀 = 1.0 and 𝜎𝑘 = 1.3

2.4.2.2.2 k-ε RNG
Although the Standard k-ε model has produced satisfactory results, its application is limited
due to uncertainties regarding turbulence production, transport and energy dissipation. K-ε
RNG and k-ε Realizable are two recent developments of the k-ε Standard model. Although kε RNG is similar to k-ε Standard model, it includes an additional term in the 𝜀 equation
80


allowing for more accurate prediction of rapidly strained and swirling flows. The equations for \( k' \) and \( \varepsilon \) are presented below:

\[
\frac{\partial}{\partial t}(\rho_L k') + \frac{\partial}{\partial x_i}(\rho_L k'U_i) = \frac{\partial}{\partial x_j}\left[(\alpha_k \mu_{\text{eff}}) \frac{\partial k}{\partial x_j}\right] + G_k + G_b - \rho_L \varepsilon - Y_M + S_k \ldots \ldots (2.21)
\]

\[
\frac{\partial}{\partial t}(\rho_L \varepsilon) + \frac{\partial}{\partial x_i}(\rho_L \varepsilon U_i) = \frac{\partial}{\partial x_j}\left[(\alpha_\varepsilon \mu_{\text{eff}}) \frac{\partial \varepsilon}{\partial x_j}\right] + C_{1\varepsilon} \frac{\varepsilon}{k'} (G_k + C_{3\varepsilon} G_b) - C_{2\varepsilon} \rho_L \frac{\varepsilon^2}{k'} - R_\varepsilon + S_\varepsilon \ldots \ldots (2.22)
\]

The additional terms \( \alpha_k \) and \( \alpha_\varepsilon \) represent the inverse effective Prandtl numbers for \( k' \) and \( \varepsilon \) respectively while the use of effective viscosity (\( \mu_{\text{eff}} \)) accounts for the model reliability in flows with lower \( Re \). At high \( Re \) the equation of turbulent viscosity is similar to eq. 2.20 with a constant \( C_\mu = 0.085 \) (ANSYS FLUENT Theory Guide Release 14.5, 2013).

### 2.4.2.2.3 k-\( \varepsilon \) Realizable

K-\( \varepsilon \) Realizable is the newest approach in k-\( \varepsilon \) models and was created to further improve the modelling of Reynolds stresses. The main difference of this model is that \( C_\mu \), which is included in the eddy viscosity equation and acts as a constant in the Standard and the RNG k-\( \varepsilon \) approaches, is now considered as a variable affected by the mean flow and turbulence. Although the equations for \( k' \) in k-\( \varepsilon \) Standard and k-\( \varepsilon \) Realizable are similar (eq. 2.18) the \( \varepsilon \) transport equation is different (eq. 2.23) as \( G_k \) term corresponding to \( k' \) generation due to velocity gradients is missing as opposed to eq. 2.19 and 2.22 for k-\( \varepsilon \) Standard and RNG respectively.

\[
\frac{\partial}{\partial t}(\rho_L \varepsilon) + \frac{\partial}{\partial x_i}(\rho_L \varepsilon U_i) = \frac{\partial}{\partial x_j}\left[(\mu + \mu_t) \frac{\partial \varepsilon}{\partial x_j}\right] + \rho_L C_1 S_\varepsilon - \rho_L C_2 \frac{\varepsilon^2}{k' + \sqrt{\varepsilon}} + C_{1\varepsilon} \frac{\varepsilon}{k'} C_{3\varepsilon} G_b + S_\varepsilon \ldots \ldots (2.23)
\]

It has been suggested that the k-\( \varepsilon \) Realizable model is the best of the aforementioned two-equation closure models outperforming them in the prediction of secondary and rotational flows (ANSYS FLUENT Theory Guide Release 14.5, 2013).

### 2.4.2.2.4 Reynolds Stress Model (RSM)

Turbulence models with two equations for the expression of turbulent kinetic energy and energy dissipation rate are widely used due to their flexibility, satisfactory accuracy in various engineering problems and low computational cost. However, those models are governed by
the assumption of isotropic and homogeneous turbulence which may affect the resulting flow profile. A key restriction of two-equation closure models is their insensitivity to accurately model streamline curvature and rotational flows. The implementation of second-order closure models, such as RSM, which use transport equations to model turbulence stresses can be used to overcome this limitation. Disregarding the hypothesis of isotropic eddy-viscosity, RSM suggests the formulation of one transport equation for each Reynolds stress resulting in seven equations for a 3D problem including six equations arising from the Reynolds stress tensor and one additional equation for energy dissipation rate (Ranade, 2002; E. Smirnov and Menter, 2009; Singh, David F. Fletcher and Nijdam, 2011; ANSYS FLUENT Theory Guide Release 14.5, 2013).

In the RSM model the individual stresses as presented in eq. 2.24 are used for closure of the time averaged momentum equation.

\[
\frac{\partial}{\partial t}(\rho U'_i U'_j) + \frac{\partial}{\partial x_k}(\rho u_k u'_i u'_j) = -\frac{\partial}{\partial x_k} [\rho U'_i u'_k + p'(\delta_{kj} u'_i + \delta_{ik} u'_j)] + \frac{\partial}{\partial x_k} [\mu \frac{\partial (U'_i U'_j)}{\partial x_k}] - \rho_i \left( U'_i u'_k \frac{\partial u'_j}{\partial x_k} + U'_j u'_k \frac{\partial u'_i}{\partial x_k} \right) - \rho_i \beta (g_i U'_i \theta + g_j U'_j \theta) + p' \left( \frac{\partial u'_i}{\partial x_j} + \frac{\partial u'_j}{\partial x_i} \right) - 2\mu \frac{\partial U'_i}{\partial x_j} \frac{\partial U'_j}{\partial x_i} - 2\rho_k \Omega_k (U'_i u'_m \epsilon_{ikm} + U'_j u'_m \epsilon_{jkm}) + S_{user} \ldots \ldots \ldots \ldots \ldots (2.24)
\]

In the above equation (eq. 2.24) the terms on the left hand side represent the local time derivative and convection whereas on the right hand side the terms symbolise the turbulence and molecular diffusion, stress and buoyancy production, pressure strain, energy dissipation, production by system rotation and a source term defined by the user. In eq. 2.24 the terms of convection, molecular diffusion, stress production and production by system rotation can be resolved without requiring modelling. However, turbulent diffusion, buoyancy production pressure strain and energy dissipation, have to be modelled in order for closure to be achieved (Ranade, Tayalia and Krishnan, 2002; ANSYS FLUENT Theory Guide Release 14.5, 2013).

**2.4.2.3 Mesh independence study**

A mesh independence study was performed by using the stirred vessel configuration B1 (Fig. 2.1). The geometry was generated in ANSYS DesignModeler (version 19.2) and separated the reactor volume into two zones: moving and stationary. The moving zone enclosed the impeller and had a height of 2w_b and a diameter of 1.6D_i. Five different unstructured mesh
densities were assessed including 250K, 600K, 800K, 900K and 1000K (1M) elements. The simulations were conducted using the Multiple Reference Frame (MRF) approach and the k-ε Realizable model while the efficacy of the grid was evaluated based on velocity and turbulence distributions (Fig. 2.7) as well as the estimated power number, \( P_0 \) (Fig. 2.8) across the different mesh densities. In Fig. 2.7(a)-(c) vertical profiles of ensemble averaged \( u_r/u_{tip}, u_z/u_{tip} \) and \( u_\theta/u_{tip} \) are illustrated for a radial distance \( r/D_T=0.24 \) in proximity to the impeller tip, at the vertical plane P1. The increase in grid density from 600K to 900K elements or more led to an increase in maximum \( u_r/u_{tip} \) and \( u_\theta/u_{tip} \) ~15% and 25% respectively while \( u_z/u_{tip} \) increased ~70% in the area below the impeller centreline. Similar trend was observed for \( k' \) and \( \epsilon \) (Fig. 2.7 (d)-(e)) with both increasing ~20% when mesh size ranged from 600K to 900K elements. Further refinement of the grid (900K elements or more) had a marginal impact on velocity distributions while \( k' \) and \( \epsilon \) changed only ~2% and 4% respectively. The impact of grid size on \( P_0 \) calculated from the torque acting on the impeller shaft (according to eq. 2.2-2.3 described in Section 2.3) is presented in Fig. 2.8. \( P_0 \) increases by ~3% when grid size was 600K-900K while further grid refinement increased \( P_0 \) by an additional ~1%. Changes in \( P_0 \) across the different grid densities were less evident compared to flow quantities, suggesting that the impact of the mesh on the moment estimation by the software is low. To confirm the validity of the aforementioned results and determine the right mesh density, hydrodynamic data were compared with published literature for radially agitated tanks (Ng et al., 1998; Delafosse et al., 2008; Steiros et al., 2017a) and \( P_0 \) was compared with published data for similar impeller types (Bates, Fondy and Corpstein, 1963). 900K elements were used to conduct a sequence of screening simulations with different RANS closure models (k-ε Standard, k-ε Realizable, k-ε RNG and RSM) to select the best precursor for LES.

### 2.4.2.4 Screening of RANS models and determination of turbulence length scales

Screening simulations have been conducted to assess the performance of the different RANS models (k-ε Standard, k-ε RNG, k-ε Realizable and RSM) and select the best one in terms of data accuracy and computational cost as the LES precursor. Vertical profiles of ensemble averaged \( u_r/u_{tip}, u_z/u_{tip}, u_\theta/u_{tip}, k'/u_{tip}^2 \) and \( \epsilon/N^3 D_i^2 \) are presented in Fig. 2.9 in close proximity to the impeller tip \( (r/D_T=0.24) \) for the bioreactor configuration with B1 at plane P1 (Fig. 2.1(b)). At \( r/D_T=0.24 \) vertical profiles of \( u_z/u_{tip} \) are similar across the different models with slightly lower values yielded by the RSM model. Similarly for \( u_r/u_{tip} \) although the trend and location of the maximum are adequately captured by all the RANS models used, lower
peak $u_r/\tilde{u}_{tip}$ is given by k-ε Realizable and RSM models (corresponding to $u_r \sim 0.5\tilde{u}_{tip}$) while a value $\sim 20\%$ higher (corresponding to $u_r \sim 0.6\tilde{u}_{tip}$) is predicted by the k-ε RNG model. Radial velocities were compared to literature for radially agitated vessels (equipped with either RTs or FBTs) at similar radial positions, which ranged within $0.6 < u_r/\tilde{u}_{tip} < 0.75$, in agreement with values predicted by the k-ε RNG model (Hartmann et al., 2004; Gimbun et al., 2012; Steiros, 2017). For $u_{\theta}/\tilde{u}_{tip}$, k-ε Standard predicted $\sim 10\%$ higher peak value at the impeller stream, compared to the rest of the RANS models used but overall all results were close to $u_{\theta}/\tilde{u}_{tip}$ published in the literature (corresponding to $u_{\theta}/\tilde{u}_{tip} \sim 0.7$) reported by (Hartmann et al., 2004; Gimbun et al., 2012). Although a baffled bioreactor configuration is discussed here, in the areas above and below the impeller region $u_{\theta}$ was approximately $0.2\tilde{u}_{tip} \pm 0.03$ suggesting the presence of solid body rotation, consistently predicted by all RANS models studied. This was estimated $\sim 10-30\%$ lower from RSM when compared to the rest of the RANS models used. The increased $u_{\theta}$ above and below the impeller can be attributed to the relatively small baffle size used ($B1=D_T/16$ being smaller than $B2=D_T/10$ which is dictated by standardised ratios of bioreactor design) and the fact the free surface deformation is not modelled and the symmetry boundary condition was defined at the top of the simulating fluid. Both parameters (i.e. baffle size and absence of simulation of free surface) will be discussed in detail in chapter 3 and assessed based on experimental validation. Nevertheless, lower $u_{\theta}$ prediction in those areas by the RSM shows lower sensitivity of the model in the deformation of the free surface. Overall, mean velocity distributions and magnitudes are equivalently predicted by the different RANS models which is in accordance with the literature as all these models are based on similar principles of the Reynolds averaging approach (Bakker et al., 1996; Montante et al., 2001; Aubin, Fletcher and Xuereb, 2004b; Bakker, 2004; Singh, Fletcher and Nijdam, 2011). Aubin et al. (2004) compared the mean flow field produced by an axial impeller (PBT) at the impeller stream in a baffled tank simulated by k-ε Standard and k-ε RNG, with LDA results and reported that mean velocities were similarly predicted by both RANS approaches and were in agreement with experimental data (Aubin, Fletcher and Xuereb, 2004b). Likewise, Sign et al. (2011) used different two-equation closure models operating under the RANS approach (including k-ε and k-ω) and compared those with subgrid models based on the estimated mean velocity field in a STR equipped with a RT. In their work it was reported that RANS approaches yielded similar results with respect to mean axial and radial velocities (Singh, Fletcher and Nijdam, 2011).
For $k'$ and $\varepsilon$, (Fig. 2.9(d)-(e)) the results generated by the different models are subject to more variations. For turbulent kinetic energy (Fig. 2.9(d)) k-\( \varepsilon \) Realizable predicts almost 15% and 50% higher $k'$ than the RSM and k-\( \varepsilon \) RNG models respectively while k-\( \varepsilon \) Standard lies in between. Moreover, maximum $k'/u_{\text{tip}}^2$ predicted by k-\( \varepsilon \) Realizable is in agreement with $k'/u_{\text{tip}}^2 \approx 0.10-0.12$ reported by (Gimbun et al., 2012) for a radially agitated vessel with a RT operating at 29,000 (V$\approx$20 L). With respect to the model comparison, similar results have been reported by Aubin et al. (2004) and Jaworski et al. (1997) after comparing k-\( \varepsilon \) Standard and RNG models in STRs equipped with a PBT and RT, respectively. Although turbulent components are expected to be underestimated by RANS models due to isotropic turbulence assumption, in their work it was stated that $k'$ produced by k-\( \varepsilon \) RNG was globally lower throughout the vessel volume suggesting that k-\( \varepsilon \) Standard would a better candidate to reproduce experimental data (Jaworski et al., 1997; Aubin, Fletcher and Xuereb, 2004b). Bakker et al.(1994) used algebraic stress model (ASM), which is a simplification of RSM, to predict mean flow and turbulence characteristics in baffled STRs mixed with both PBT and RT. In their work it was reported that the ASM performance outweighed k-\( \varepsilon \) Standard in the prediction of root mean squared (rms) velocity components and turbulence in different location of the tank, which is in accordance with Fig. 2.9(d) when comparing k-\( \varepsilon \) Standard and RSM (Bakker and den Akker, 1994; Joshi et al., 2011b). Similar trends were predicted for $\varepsilon$ (Fig. 2.9(e)), with k-\( \varepsilon \) RNG estimating the lowest and k-\( \varepsilon \) Realizable and RSM models the highest values across all the RANS models examined. However, the double peak in both vertical profiles of $k'/u_{\text{tip}}^2$ and $\varepsilon/N^3D_i^2$ in the impeller stream region (0.3$<z/D_T$<0.4) is predicted by k-\( \varepsilon \) RNG, which is characteristic of the trailing vortex formation. This trend is not predicted by any other model studied and could be attributed to its higher capacity to model high swirling flows (Aubin, Fletcher and Xuereb, 2004a). Volume averaged values of $\bar{\varepsilon}/N^3D_i^2$ have been computed for the different RANS models and compared to computational results of Delafosse et al. (2008), obtained for a radially agitated STR with a RT, V$\approx$70 L operating at Re$\approx$55,000, and are summarised in Table 2.6 (Delafosse et al., 2008). According to Table 2.6, lower mean value of $\bar{\varepsilon}/N^3D_i^2$ was estimated from the k-\( \varepsilon \) RNG model and higher from the RSM while for k-\( \varepsilon \) Realizable volume averaged $\bar{\varepsilon}/N^3D_i^2$ is similar to that one reported by (Delafosse et al., 2008). The aforementioned analysis and data comparison was conducted to assess the validity of RANS modelling and compare the different models. Differences in bioreactor working volumes, impeller configurations and flow regime, should be taken into account for an in-depth characterisation of velocity and turbulence quantities as discrepancies could be attributed to design and operating conditions.
The different RANS models used were compared based on the $P_0$ and results are presented in Table 2.7. $P_0$ was calculated according to eq. 2.2-2.3 based on the estimated moment of the impeller and shaft from each model. The variation of $P_0$ across the different models was approximately 2-6% while the estimated $P_0$ for FBT based on published literature is ~4.7-5, diverging only ~2-8% from 4.7 and 4.6 which were the values predicted by k-$\epsilon$ Realizable and RSM, respectively (Bates, Fondy and Corpstein, 1963). Further analysis of $P_0$ is conducted in chapter 5. The RSM, by explicitly computing Reynolds stresses and neglecting isotropic assumption for turbulence, is capable of extensively resolving the flow compared to other RANS models while less flow parameters are modelled. However, this exponentially increases the computational cost and often introduces convergence issues that render RSM the least used model across RANS approach (Aubin, Fletcher and Xuereb, 2004b). For this reason, in the current work k-$\epsilon$ Realizable was selected to be used as a precursor for LES as it was the second best performing model with respect to turbulence prediction and average flow field.

All screening simulations were performed with a grid size of 900K elements using the SM approach to account for impeller rotation and transient interactions between impeller and baffles (Aubin, Fletcher and Xuereb, 2004b; M Coroneo et al., 2011; Singh, David F. Fletcher and Nijdam, 2011). The time step used corresponded to 1° angular displacement ($dt=6.66 \times 10^{-4}$ s) that kept cell convective courant number less than 1 for the entire volume of the tank (Delafosse et al., 2008). According to the predicted average dissipation rate, $\epsilon$, and turbulent kinetic energy, $k'$, integral, Taylor and Kolmogorov length scales were estimated based on eq. (2.25-2.27) and their orders of magnitude were $\overline{l_o} \propto 10^{-3}$m, $\overline{\lambda} \propto 10^{-4}$m and $\overline{\eta} \propto 10^{-5}$ m, respectively (Escudié and Liné, 2003; Ducci and Yianneskis, 2005a).

\[
l_0 = \frac{k^{1.5}}{\epsilon} \hspace{2cm} \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.25)
\]

\[
\lambda = \sqrt{\frac{15 \nu \overline{u'^2}}{\epsilon}} = \sqrt{\frac{10 \nu k}{\epsilon}} \hspace{2cm} \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.26)
\]

\[
\eta = \left(\frac{\nu^3}{\epsilon}\right)^{0.25} \hspace{2cm} \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.27)
\]

Average values of integral and Taylor length scales were used to pick an adequate grid spatial resolution for the conduction of LES. Maximum energy dissipation rate, $\epsilon_{max}$, was also used to compute the Kolmogorov timescale (eq. 2.11) and therefore refine the temporal resolution.
of the LES in the impeller region (Collignon et al., 2016). The simulation conditions and the different approaches used are summarised in Table 2.8.

Double precision was used to perform all RANS simulations. A pressure-based implicit solver was applied and a second order upwind scheme was used for spatial discretisation of continuity and momentum equations. SIMPLEC algorithm was used for pressure-velocity coupling. Convergence was evaluated based on residuals being less than $10^{-6}$ for continuity and momentum and $10^{-5}$ for turbulence. Other physical convergence criteria included monitoring the velocity at different points in the vessel, average turbulence in the impeller rotational area and the momentum of the shaft.

2.4.2.5 LES modelling
The second set of CFD simulations was conducted using LES. When using LES, the Navier-Stokes equations are filtered and solved up to large turbulence scales within a certain cut-off grid size, beyond which smaller turbulence scales (subgrid scales) are assumed to be isotropic and are modelled via a subgrid scale model (eq. 2.28-2.29). The filtered Navier-Stokes equations are:

\[
\frac{\partial \bar{u}_i}{\partial x_i} = 0 \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.28)
\]

\[
\frac{\partial \bar{u}_i}{\partial t} + \frac{\partial}{\partial x_j}(\bar{u}_i \bar{u}_j) = -\frac{1}{\rho} \frac{\partial p}{\partial x_i} + \nu \frac{\partial^2 \bar{u}_i}{\partial x_j^2} - \frac{\partial \tau_{ij}^{SGS}}{\partial x_j} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.29)
\]

\[
\tau_{ij}^{SGS} = -\nu_T \left( \frac{\partial \bar{u}_i}{\partial x_j} + \frac{\partial \bar{u}_j}{\partial x_i} \right) \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.30)
\]

\[
\nu_T = C_s \Delta \sqrt{\frac{1}{2} \left( \frac{\partial \bar{u}_i}{\partial x_i} + \frac{\partial \bar{u}_j}{\partial x_j} \right)} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.31)
\]

In this work, the Smagorinsky model was used which is an eddy-viscosity model relating the subgrid-eddy viscosity, $\nu_T$, to the resolved deformation rate (eq. 2.31). The filtering process in Fluent is determined from the grid spacing, $\Delta$, therefore turbulence scales larger than the grid size are fully resolved, whereas smaller turbulence scales are modelled. The Smagorinsky constant used in this study was $C_s=0.1$, which is typical for turbulent flows (Smagorinsky, 1963) and in agreement with previous studies of Delafosse et al. (2009), who did not find significant variation in velocity and turbulent kinetic energy distribution between $C_s=0.1$ and $C_s=0.2$ (Delafosse et al., 2008). The selected value of $C_s$ is also in agreement with Gillisen et al.
(2012) who compared results between LES and Direct Numerical Simulations (DNS) and found out that $C_s = 0.1$ was suitable for both mean velocity and turbulence prediction (Gillissen and den Akker, 2012).

After mesh refinement based on the estimated Taylor microscale, the final grid used for LES consisted of 3 million tetrahedral elements with spatial resolution $7 \mu m < \Delta x < 1.8 \text{ mm}$ (Fig. 2.10). In the refined grid $P_0$ was calculated based on the moment acting on the impeller shaft, and plotted in Fig. 2.11 together with all the grid densities used previously (280K-1 million elements) in order to identify discrepancies arising from the mesh refinement. The estimated values equalled 4.7 and 4.8, corresponding to $\sim 2\%$ increase between 900K and 3 million elements confirming again that the estimation of moment (based on which $P_0$ is calculated) is not sensitive on grid density. In agreement with Hartmann et al. (2004) the ratio of eddy viscosity over kinematic viscosity ($\nu_T/\nu$) was monitored during the course of the simulation to ensure that the grid quality was good enough to successfully resolve a large range of turbulence length scales and minimise the amount of modelling occurring within the subgrid scale (Hartmann et al., 2004). The resulting ratio in the sliding mesh region had an average value of $\nu_T/\nu \sim 20\%$ indicating adequate spatial resolution as almost 80% of the flow field was appropriately resolved and only 20% was modelled.

SIMPLEC algorithm was used for pressure-velocity coupling and Bounded Central Differencing scheme for spatial discretisation of momentum. Convergence was monitored based on residual values being less than $10^{-5}$ for momentum equations, while additional criteria included the statistical convergence of velocity magnitude in points in and out of the rotational area. Impeller momentum was also monitored during the course of LES to ensure convergence was reached. The time step size was selected to be $dt = 3.3386 \times 10^{-4}$ s, lower than the Kolmogorov timescale estimated from URANS simulations ($\tau_K = (\nu/\varepsilon)^{0.5} \times 10^{-3}$ s) corresponding to an angular displacement of $d\phi \sim 0.5^\circ$ (Fig. 2.12) and ensuring the Courant number was below unity in the whole reactor volume. Forty (40) revolutions have been simulated for the UB and the baffled configurations equipped with B2, and eighty (80) revolutions for the baffled case equipped with B1. For all configurations the first 20 revolutions were performed to ensure simulation statistical convergence and the remaining 20 (UB and baffled with B2), and 60 revolutions (baffled with B1), were used for data acquisition and processing. This corresponds to 14,400 instantaneous acquisitions for the UB and the B2 vessel configurations. For the baffled configuration B1, the simulation time was
extended to 60 revolutions (~43,200 acquisitions) as this was the standard vessel configuration designed and tested by (Tregidgo, 2021).

2.4.3 Processing of computational and experimental flow data

The post-processing of calculated velocity fields for both simulation and experiments was conducted in MATLAB (R2018a). For CFD results, a regular space grid of \([dx, dz] = 0.7\) mm was used to map the unstructured computational grid and its order of magnitude was consistent with the grid originated experimentally. The generated grid agreed with the minimum cell size from ANSYS meshing and was of the same order of magnitude of the average estimated Taylor microscale.

LES phase resolved velocity fields were estimated from 120 and 360 instantaneous fields (simulation of 20 and 60 impeller revolutions respectively) corresponding to blade positions of \(\varphi=0^\circ-60^\circ\). For example, for B1 configuration for which 60 impeller revolutions have been simulated, the phase resolved velocity field at \(\varphi=10^\circ\) was calculated by using a screenshot of the instantaneous \((u_i(\varphi,t))\) velocity every blade passage, i.e. 60\(^\circ\) or 120 time steps. This corresponded to six samples for each revolution or 360 samples for 60 revolutions.

\[
\langle u_i \rangle = \sum_{i=1}^{i=n_\varphi} \frac{u_i(\varphi,t)}{n_\varphi} ........................................(2.32)
\]

where \(n_\varphi\) is the number of samples corresponding to a certain phase angle \(\varphi\) (500 for PIV and 120-360 for LES depending on the number of impeller revolutions). Ensemble averages for both experiments and simulations were obtained by phase resolved data averages as follows:

\[
u_i = \sum_{i=1}^{i=n} \frac{\langle u_i \rangle}{n} ........................................(2.33)
\]

where \(n\) is the number of angles (\(\varphi\)) for which phase resolved data have been obtained. For LES, data exist over all impeller positions (i.e. 0-60\(^\circ\), with a temporal resolution corresponding to angular displacement of 0.5\(^\circ\)) thus \(n=120\). For PIV phase resolved data were obtained from 0\(^\circ\) to 60\(^\circ\) every 5\(^\circ\), therefore \(n=12\).

Calculations of velocity magnitude were conducted according to eq. 2.34 where \(i, j\) corresponded to axial and radial (\(u_z\) and \(u_r\) respectively) or radial and tangential (\(u_r\) and \(u_\theta\) respectively) velocity components, depending on whether the plane of measurement was vertical or horizontal.
\[ |u| = \sqrt{(u_i)^2 + (u_j)^2} \]  

(2.34)

Tangential and axial vorticity, \(\omega_\theta\) and \(\omega_z\), were calculated based on central differencing scheme as follows:

\[
\omega_\theta = \frac{\partial u_r}{\partial z} - \frac{\partial u_z}{\partial r} \]  

(2.35)

\[
\omega_z = \frac{1}{r} \frac{\partial (ru_\theta)}{\partial r} - \frac{1}{r} \frac{\partial u_r}{\partial \theta} \]  

(2.36)

The velocity field in the impeller region can be characterised by periodic fluctuations, arising from the trailing vortices, and random fluctuations coming from turbulent motions. The instantaneous velocity field can be decomposed according to eq. 2.37:

\[
u_i(\varphi, t) = u_i + \tilde{u}_i + u'_i \]  

(2.37)

where \(u_i\), \(\tilde{u}_i\) and \(u'_i\) represent the mean, periodic and turbulent velocity components, respectively. Similarly, the total kinetic energy of the system can be decomposed into periodic and turbulent (eq. 2.38) that can be calculated from the individual velocity components, \(\tilde{u}_i\) and \(u'_i\) respectively (eq. 2.39-2.40).

\[
k_{tot} = k_{per} + k' \]  

(2.38)

\[
k_{per} = 0.5 \cdot \left( \tilde{u}_r^2 + \tilde{u}_\theta^2 + \tilde{u}_z^2 \right) \]  

(2.39)

\[
k' = 0.5 \cdot \left( u'_r^2 + u'_\theta^2 + u'_z^2 \right) \]  

(2.40)

In eq. 2.39 \(\tilde{u}_r, \tilde{u}_\theta\) and \(\tilde{u}_z\) can be calculated from the phase resolved velocities, \(\langle u_i \rangle\), in each direction \((r, \theta, z)\) and the mean velocity \(u_i\) as follows:

\[
\tilde{u}_i^2 = \frac{1}{N} \sum_{i=1}^{i=N} \left( \langle u_i \rangle - u_i \right)^2 \]  

(2.41)

In eq. 2.41, \(u'_i\) (where \(i=r, \theta, z\)) corresponds to the root mean square (rms) velocity and measures the velocity fluctuations (eq. 2.42).

\[
u'_i = \frac{1}{n_p} \sum_{i=1}^{i=n_p} \left( u_i(\varphi, t) - \langle u_i \rangle \right)^2 \]  

(2.42)
Given the 2D nature of the PIV system used in the present work, the contribution to the turbulent kinetic energy due to velocity fluctuations in the tangential and axial directions for the vertical and horizontal planes, respectively, were included in the estimation of $k'$ by assuming pseudo-isotropic flow (eq. 2.43).

$$k' = 0.75 \cdot (u'^2_r + u'^2_z) \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.43)$$

This is consistent with the works of Zhao et al. (2011) and Khan et al. (2006), who demonstrated that this assumption is valid for fully baffled stirred tank reactor flows and turbulent kinetic energy estimates are only marginally affected (Khan, Rielly and Brown, 2006; Zhao, Gao and Bao, 2011). Turbulent kinetic energy was calculated by subtracting the phase resolved velocity $\langle u_i \rangle$ from the instantaneous velocity $(u_i(\varphi, t))$ at the same blade position. Average $k'$ was obtained by averaging the corresponding phase resolved values from PIV and LES datasets, with $5^\circ$ and $0.5^\circ$ resolution respectively, obtained based on eq. 2.42.

### 2.4.4 Proper Orthogonal Decomposition (POD)

POD has been employed to identify dominant frequencies and flow structures in a broad range of flow fields (de Lamotte et al., 2018a; Weheliye et al., 2018). It is a linear technique which allows to decompose an instantaneous flow snapshot into a number of spatial and temporal modes of descending kinetic energy magnitude. The first modes therefore contain most of the kinetic energy embedded in the flow and are linked to larger scale structures, as opposed to lower order modes which have less kinetic energy and are associated with smaller scales and turbulence. Once dominant modes are identified, POD allows to produce low order models (LOMs) which can be used to substantially reduce the dimension of the dataset, and therefore isolate flow instabilities (Ducci, Doulgerakis and Yianneskis, 2008; Weheliye et al., 2018). In this work, POD was used with the method of snapshot, with matrix $X$ being characterised by $R$ rows, corresponding to spatial variation in the radial and axial directions or radial and tangential directions for the analysis of vertical and horizontal planes respectively, and $M$ columns, corresponding to the number of frames used either from the PIV experiments or LES (eq. 2.44).

$$X = \begin{bmatrix} u_i(x_1, t_1) & \ldots & u_i(x_1, t_M) \\ \vdots & \ddots & \vdots \\ u_i(x_R, t_1) & \ldots & u_i(x_R, t_M) \\ u_j(x_1, t_1) & \ldots & u_j(x_1, t_M) \\ \vdots & \ddots & \vdots \\ u_j(x_R, t_1) & \ldots & u_j(x_R, t_M) \end{bmatrix} \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.44)$$
When using the POD modes an instantaneous velocity field, \( \mathbf{u}_i \), can be written as follows (eq. 2.45):

\[
\mathbf{u}_i(x,t) = \sum_{n=1}^{n=M} a_n(t) \Phi_n(x) \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots} \]

where \( a_n(t) \) is the temporal coefficient and \( \Phi_n(x) \) is the spatial eigenfunction associated with the \( n \)th mode. More details on the method can be found in the literature (Sirovich, 1987; Ducci, Doulgerakis and Yianneskis, 2008; Liné, 2016; de Lamotte et al., 2018b; Weheliye et al., 2018).

2.5 Summary

In this chapter the bioreactor configurations used for computational and experimental investigation of the flow dynamics were described. The parameters examined for the tuning of CFD simulations were detailed including mesh independence studies and RANS model screening to identify the best precursor for the conduction of LES. Overall, all the RANS models used, including \( k-\varepsilon \) Standard, \( k-\varepsilon \) RNG, \( k-\varepsilon \) Realizable and RSM led to similar predictions with respect to the average velocity field and followed satisfactorily the literature data for fully turbulent flows in STRs. Larger deviations across the different RANS models appeared with respect to turbulence quantities (i.e. \( k' \) and \( \varepsilon \)) with \( k-\varepsilon \) RNG yielding 60-80\% lower values compared to \( k-\varepsilon \) Realizable for \( \varepsilon \) and \( k' \) respectively. Power number analysis indicated that the \( P_0 \) values estimated from the moment acting on the impeller shaft were robust and were not affected significantly either by the mesh density (3-4\% difference across the different mesh densities studied) or the RANS model (2-6\% difference across the different RANS models studied) used, while predictions were in agreement with published literature. Due to better prediction of turbulence quantities and the relatively low computational cost, \( k-\varepsilon \) Realizable model was selected to be used as a precursor for LES. The latter were conducted in high spatial and temporal resolution, determined by the length and time scales estimated by the RANS simulations.

The experimental methods implemented in the current work were summarised, including PIV technique used for CFD validation and methods for the measurement of power consumption, alongside potential sources of error which were considered and minimised. Finally, post-processing methodologies for the analysis and interpretation of experimental and computational results were described. In the next chapters, the aforementioned techniques are implemented for an end-to-end characterisation of flow and power characteristics in SDMs used in pharmaceutical process development.
Figure 2.1: Bioreactor configuration: (a) acrylic mimic of the 250 mL bioreactor used in biological experiments by Tregidgo, (2021), (b) schematic representation of the vessel presented in (a) alongside its sizing parameters, (c) baffled configuration with B1 equipped with a Rushton Turbine (RT) and (d) baffled configuration with B1 equipped with two probes for oxygen and pH monitoring in two different sizes. All the vessel configurations were designed on Ansys DesignModeler. For all configurations $D_T=67$ mm and corresponds to the internal diameter of the vessel.
Figure 2.2: Experimental setup of the 60 mL EasyMax™ (Mettler Toledo) equipped with a 45° PBT. For this configuration $D_T=52$ mm and corresponds to the internal diameter of the vessel.
Figure 2.3: (a) Configuration of the acrylic mimic of the commercially available 1 L Univessel® (Sartorius) Glass bioreactor equipped with a 45° 3BS impeller and a FBT and (b) the novel probes used. Details about sizing characteristics of the vessel and the 45° 3BS impeller can be found at Wyrobnik et al., (2021). For this configuration $D_T = 110$ mm and corresponds to the internal diameter of the vessel.
Table 2.1: Summary of bioreactor configurations used, geometry and agitation type. For all the configurations UB/NI corresponded to V(%)=0 blockage. Details about sizing characteristics of the vessel of Fig. 2.3 and the 45° 3BS impeller can be found at Wyrobnik et al., (2021).

<table>
<thead>
<tr>
<th>Bioreactor Configuration</th>
<th>Working volume (L)</th>
<th>Impeller type</th>
<th>Impeller sizing</th>
<th>( \frac{C}{D_l} )</th>
<th>Baffle presence (UB/B)/Baffle size (B/D_l)</th>
<th>Internal presence (NI)/Blockage ratio (%)</th>
<th>Probe volume</th>
<th>Working volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Custom made bioreactor for mammalian cell processing UCL</td>
<td>0.25</td>
<td>FBT, RT</td>
<td>( \frac{D_l}{D_T} = 0.44 ); ( D_T = 67 \text{ mm} )</td>
<td>( \frac{t_{\text{blade}}}{D_l} = 0.05 ); ( \frac{t_{\text{blade}}}{D_l} = 0.33 ) (RT)</td>
<td>UB, B1: ( B/D_T = B1/D_T = 0.06 ); B2: ( B/D_T = B2/D_T = 0.1 )</td>
<td>V(%)~0 (UB/NI)-2.8 (2xPa); V(%)~0 (UB/NI)-6.3 (2xPa)</td>
<td>2 probes of equal size</td>
<td></td>
</tr>
<tr>
<td>EasyMax™ Mettler-Toledo</td>
<td>0.06</td>
<td>45° PBT</td>
<td>( \frac{D_l}{D_T} = 0.5 ); ( D_T = 52 \text{ mm} )</td>
<td>( \frac{t_{\text{blade}}}{D_l} = 0.12 )</td>
<td>UB, ( B/D_T = 0.1 )</td>
<td>V(%)~0 (UB/NI)-8.4 (max blockage)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Univessel® Sartorius</td>
<td>1</td>
<td>45° 3BS, FBT</td>
<td>( \frac{D_l}{D_T} = 0.44 ); ( D_T = 110 \text{ mm} )</td>
<td>( \frac{t_{\text{blade}}}{D_l} = 0.03 ) (3BS); ( \frac{t_{\text{blade}}}{D_l} = 0.04 ) (FBT)</td>
<td>UB</td>
<td>V(%)~0 (UB/NI)-3 (max blockage) – culture probes (3BS); V(%)~0.2-1 – novel probes (FBT)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.2: Summary of characteristics of bioreactor internals, addition lines and probes, used for the different STRs.

<table>
<thead>
<tr>
<th>Bioreactor Configuration</th>
<th>Internal (Line L, Probe P) diameter ( (D_L / D_T, DP / D_T) )</th>
<th>Internal (Line L, Probe P) length ( L_L / H_L, L_P / H_L )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Custom made bioreactor for mammalian cell processing UCL</td>
<td>*2 probes of similar size per run&lt;br&gt;*2 different sizes examined&lt;br&gt;• P(<em>{pH}), P(<em>T): ( D</em>{p} / D_T =0.12 )&lt;br&gt;• P(</em>{pH}), P(<em>T): ( D</em>{p} / D_T =0.18 )</td>
<td>• ( L_{pH} / H_L =0.9 )&lt;br&gt;• ( L_{TP} / H_L =0.9 )&lt;br&gt;• all probes have the same length</td>
</tr>
<tr>
<td>EasyMax™ Mettler-Toledo</td>
<td>• ( D_{pPH} / D_T =0.23 )&lt;br&gt;• ( D_{PT} / D_T =0.06 )&lt;br&gt;• ( D_{L1} / D_T =0.06 )&lt;br&gt;• ( D_{L2} / D_T =0.04 )</td>
<td>• ( L_{pPH} / H_L =0.86 )&lt;br&gt;• ( L_{PT} / H_L =0.78 )&lt;br&gt;• ( L_{L1} / H_L =0.58 )&lt;br&gt;• ( L_{L2} / H_L =0.38 )</td>
</tr>
<tr>
<td>Univessel® Sartorius</td>
<td>Culture probes: ( 0.05 &lt; \frac{D_P}{D_T} &lt; 0.1 )&lt;br&gt;Novel probes: N/A</td>
<td>Culture probes: ( 0.5 &lt; \frac{L_P}{H_L} &lt; 0.8 )&lt;br&gt;( \frac{LN}{H_L} =0.8 )</td>
</tr>
</tbody>
</table>
Table 2.3: Summary of the type of analysis conducted with each bioreactor configuration used in the current work. The studies conducted in each configuration are denoted with “x”.

<table>
<thead>
<tr>
<th>Bioreactor Configuration</th>
<th>Working volume (L)</th>
<th>Impeller type</th>
<th>Presence of baffles and/or internals</th>
<th>Flow analysis</th>
<th>Power consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Custom made bioreactor for mammalian cell processing UCL</td>
<td>0.25</td>
<td>• FBT • RT</td>
<td>• UB/NI  • B1  • B2  • P_{pH}, P_{O_2}; Pa  • P_{pH}, P_{O_2}; Pb  • P_a+B1  • P_b+B1</td>
<td>CFD</td>
<td>N/A</td>
</tr>
<tr>
<td>EasyMax™ Mettler-Toledo</td>
<td>0.06</td>
<td>45° PBT</td>
<td>• UB/NI  • B  • P_{pH}  • P_a  • L1+L2  • P_{pH}+P_a  • P_{pH}+P_a+L1+L2</td>
<td>N/A</td>
<td>x</td>
</tr>
<tr>
<td>Univessel® Sartorius</td>
<td>1</td>
<td>• 45° 3BS • FBT</td>
<td>• UB/NI  • Culture probes (45° 3BS)  • Novel probes (FBT)</td>
<td>N/A</td>
<td>x</td>
</tr>
</tbody>
</table>
Figure 2.4: Experimental setup for power number measurements: (a) actual experimental apparatus used and (b) schematic of the experimental setup.
Figure 2.5: Experimental setup PIV experiments used for the validation of CFD simulations: (a) picture and (b) schematic diagram of the experimental setup; (c) the vertical planes of measurement; (d) the horizontal planes of measurement.
Table 2.4: Experimental parameters used in the PIV experiments. The shaft speed was always $N = 4.16$ 1/s corresponding to $Re \approx 3,732$.

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Acquisition Frequency (Hz)</th>
<th>Resolution (Pixels)</th>
<th>Experimental Spatial resolution (mm)</th>
<th>Vector count</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB/B2</td>
<td>4000</td>
<td>460x260</td>
<td>0.5</td>
<td>32x57</td>
</tr>
<tr>
<td>B1</td>
<td>2000</td>
<td>470x510</td>
<td>1.1</td>
<td>31x28</td>
</tr>
</tbody>
</table>
Figure 2.6: Statistical convergence of velocity magnitude for PIV experiments at three different points in the bioreactor: (a) time and (b) phase resolved measurements (corresponding to $\varphi=30^\circ$). Points A, B and C are indicated for the plane of measurement $P_1$ for the baffled configuration with $B_1$ (Fig. 2.1).
Table 2.5: Summary of the characteristics of the RANS models used (k-ε and RSM) during screening simulations.

<table>
<thead>
<tr>
<th>RANS model</th>
<th>Number of equations</th>
<th>Characteristics</th>
<th>Cited literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>• k-ε Standard</td>
<td>2:</td>
<td>• Satisfactory results for the mean flow field in fully turbulent conditions with low computational cost</td>
<td>(Delafosse et al., 2008)</td>
</tr>
<tr>
<td>• k-ε Realizable</td>
<td></td>
<td>• Robustness in modelling of hydrodynamics in STRs</td>
<td>(Collignon et al., 2016)</td>
</tr>
<tr>
<td>• k-ε RNG</td>
<td></td>
<td>• Turbulence isotropy</td>
<td>(Zdzislaw Jaworski et al., 1997; Aubin, Fletcher and Xuereb, 2004b)</td>
</tr>
<tr>
<td>RSM</td>
<td>7</td>
<td>• Solution of transport equations for Reynolds stresses without the isotropic hypothesis</td>
<td>(Bakker and den Akker, 1994; Joshi et al., 2011a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Accounts for streamline curvature and swirling flows</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Limited application due to high computational cost and limited confidence that ε and pressure-strain are correctly simulated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 equations for each Reynolds stress at the Reynolds stress tensor</td>
<td>• 1 equation for ε</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.7: Results for mesh independence study for the B1 configuration (Fig. 2.1 (b)) for velocity and turbulence distributions: (a) $u_r / u_{tip}$ , (b) $u_z / u_{tip}$ , (c) $u_\theta / u_{tip}$ , (d) $k'/u_{tip}^2$ and (e) $\varepsilon / (N^3D_f^2)$. Curves are presented at $r/D_r \sim 0.24$ for all the different mesh densities on plane $P_1$. 

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Figure 2.8: $P_0$ estimated for the different grid sizes (250K-1 million) for the B1 configuration (Fig. 2.1 (b)) using the MRF approach and the $k$-$\varepsilon$ Realizable model.
Figure 2.9: Results for RANS models screening for the B1 configuration (Fig. 2.1 (b)) for velocity and turbulence distributions: (a) $u_r / u_{tip}$, (b) $u_z / u_{tip}$, (c) $u_\theta / u_{tip}$, (d) $k'/u_{tip}^2$, and (e) $\epsilon / (N^3D_i^2)$. Curves are presented at $r/D_T \sim 0.24$ for all the different RANS models studied, at the vertical plane $P_1$. 
Table 2.6: Overall energy dissipation rate ($\bar{\varepsilon}$) predicted by different RANS models in the current work for baffled vessel with B1 (Fig. 2.1 (b)) and comparison with computational results published in the literature.

<table>
<thead>
<tr>
<th></th>
<th>k-$\varepsilon$ Standard</th>
<th>k-$\varepsilon$ RNG</th>
<th>k-$\varepsilon$ Realizable</th>
<th>RSM</th>
<th>Delafosse et al. (2008)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{\varepsilon}/N^3D_i^2$</td>
<td>0.23</td>
<td>0.22</td>
<td>0.26</td>
<td>0.27</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Table 2.7: $P_0$ estimation for baffled vessel with B1 (Fig. 2.1 (b)) configurations after simulation screening with different RANS models. $P_0$ was calculated from the torque applied on the impeller shaft.

<table>
<thead>
<tr>
<th>model</th>
<th>$P_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>k-$\varepsilon$ Standard</td>
<td>4.8</td>
</tr>
<tr>
<td>k-$\varepsilon$ Realizable</td>
<td>4.7</td>
</tr>
<tr>
<td>k-$\varepsilon$ RNG</td>
<td>4.9</td>
</tr>
<tr>
<td>RSM</td>
<td>4.6</td>
</tr>
</tbody>
</table>
Figure 2.10: Illustration of mesh density before and after refinement. On the left hand side the grid of 900K elements used for the conduction of screening simulations is illustrated. On the right hand side the mesh consisting of 3 million elements used in LES is presented after refinement based on Taylor microscale, as described in Section 2.4.2.3.
Figure 2.11: $P_0$ for the different grid sizes (250K-3 million) for the baffled configuration with B1 (Fig. 2.1 (b)) estimated with MRF and k-ε Realizable model.
Table 2.8: Summary of the different conditions used across the different steps of the simulation process.

<table>
<thead>
<tr>
<th>CFD simulation</th>
<th>Steady state (MRF)/ Transient simulation (Sliding Mesh)</th>
<th>Mesh size (number of elements)</th>
<th>Model</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANS</td>
<td>Steady state (MRF)</td>
<td>250-1000 K</td>
<td>k-ε Realizable</td>
<td>Mesh independence</td>
</tr>
<tr>
<td>URANS</td>
<td>Transient (Sliding Mesh)</td>
<td>900K</td>
<td>k-ε Standard, k-ε Realizable, k-ε RNG, Reynolds Stress Model (RSM)</td>
<td>Screening of URANS models</td>
</tr>
<tr>
<td>URANS</td>
<td>Transient (Sliding Mesh)</td>
<td>3M</td>
<td>k-ε Realizable</td>
<td>LES precursor</td>
</tr>
<tr>
<td>LES</td>
<td>Transient (Sliding Mesh)</td>
<td>3M</td>
<td>Smagorinsky model (C_s = 0.1)</td>
<td>LES simulation/results extraction</td>
</tr>
</tbody>
</table>
Figure 2.12: Illustration of the time resolution and angular displacement used in LES. The time step size corresponded to $3.886 \times 10^{-4}$ s which is equivalent to 120 angular positions for one impeller revolution, each corresponding to 0.5°.
Chapter 3: Bioreactor hydrodynamics analysis: CFD results and experimental validation

3.1 Introduction

The industrial interest towards the use of small scale bioreactors (mL-scale) in process development is increasingly growing as these devices can facilitate automated operation and allow parallel experimental runs assisting in the understanding of process development. Small scale bioreactors have been developed to replicate the culture conditions at larger vessels aiming at reducing the discrepancies in bioperformance across different scales (Nienow, Rielly, et al., 2013a). Although to date there are several small scale bioreactors that are commercially available, the data from engineering characterisation is rare and limited to larger scale systems corresponding to several litres. Nevertheless, matching of (bio)chemical processes among small and larger vessels may result in altering critical operating parameters in the former (e.g. increase in rotational speed, changes in flow regime), leading to lack of dynamic similarity across the different volumes which often influences the overall flow and mixing performance (Nienow, Rielly, et al., 2013a; Xu et al., 2017). Evaluation of the engineering characteristics in small scale STRs is particularly important for the development of robust scale-down models (SDMs) and a thorough understanding of the hydrodynamics is essential to evaluate the way critical design parameters affect the flow patterns, assisting in the optimisation of the mixing and cell growth processes.

* Some of the results presented in this chapter are included in:


Charalambidou, A. D., Ducci, A., Micheletti, M. (2021) “Numerical and experimental investigation of the flow generated by a flat blade impeller used in perfusion processes”, 10th International Symposium in Mixing in Industrial Processes, Kobe, Japan

Fluid flow in industrial stirred tank reactors (STRs) can be characterised by a time averaged component and a fluctuating component. The former is associated with the presence of several recirculation loops the structure, intensity and position of which can be affected by the vessel geometry and type of agitation. The latter depends on the operating flow regime and the velocity gradients generated during impeller rotation and determines the levels of shear that would eventually impact process performance.

In this chapter investigation of hydrodynamics was performed in a custom made SDM of 250 mL successfully used in biological studies of antibody producing CHO cell cultures in perfusion mode (Tregidgo, 2021). The bioreactor is presented in Fig. 2.1(a) and Fig. 2.1(b) and was radially agitated with a six-blade Flat Blade Turbine (FBT). To investigate the impact of baffles presence and size on the mean flow characteristics three bioreactor configurations have been used: one unbaffled (UB) and two baffled configurations equipped with three baffles of increasing width, \( B_1 = \frac{D_T}{16} \) and \( B_2 = \frac{D_T}{10} \). Characterisation of the mean flow was performed by Computational Fluid Dynamics (CFD) with k-\( \varepsilon \) Realizable model operating under the RANS approach and LES, on a grid consisting of 3 million elements, as described in chapter 2 (Section 2.4.2.3-2.4.2.5). Model performance was assessed and validated with Particle Image Velocimetry (PIV) experiments for all the bioreactor configurations studied (UB, B1 and B2).

### 3.2 Results

The LES velocity fields were validated against the corresponding PIV results for all the configurations investigated (UB, B1 and B2). LES and PIV results are presented side-by-side in this chapter for the purpose of validation and to shed light on the impact of baffles on the average velocity field for the radial, axial and tangential velocity components, on the phase-resolved velocity and on the turbulent kinetic energy distribution.

#### 3.2.1 Impact of baffles on average velocity field

A direct comparison between the velocity characteristics of the three configurations, UB, B1 and B2 on a vertical plane is presented in Fig. 3.1 and Fig. 3.2 by means of contour and vector plots, respectively. Contour maps of ensemble average velocity magnitude, \( \frac{|u|}{u_{tip}} \), are presented in Fig. 3.1 for both experiments and simulations on a vertical plane (\( P_1 \) for the baffled vessels) and it can be observed that the qualitative agreement between PIV and LES is good both in terms of magnitude and vector distribution. Fig. 3.1 illustrates that the in-plane velocity magnitude increases with the presence of baffles and with baffle size. This is expected.
as for UB vessels the flow is close to solid body rotation and the most significant velocity component under this condition is the tangential one, which is not accounted for in the contours presented in Fig. 3.1. The impact of baffles on the main circulation loops of the flow is shown in Fig. 3.2 where vector plots of the ensemble average velocity field are presented for 90% of the total liquid height \((0<z/D_T<0.9)\) based on LES results for the same vertical plane \((P_1)\). The upper circulation loop for the unbaﬄed case reaches a maximum at approximately \(r/D_T\sim0.7\) while it reaches \(r/D_T\sim0.85\) and \(r/D_T\sim0.9\) when baffles are present, for B1 and B2, respectively. This conﬁrms that baffles transfer energy from the tangential to the axial and radial velocity components, resulting in larger and more effective circulation loops.

### 3.2.1.1 Time-resolved velocity field distribution

Vertical proﬁles of ensemble averaged radial \((u_r/u_{tip})\) and axial \((u_z/u_{tip})\) velocity components are presented in Fig.3.3, Fig. 3.4 and Fig. 3.5 for the three vessel conﬁgurations studied at two radial distances: \(r/D_T=0.25\), close to the impeller tip where maximum velocities are expected to occur, and \(r/D_T=0.35\).

The agreement between experiments and LES is good with the axial velocity component having an error of \(~4\%\) for the UB case and \(~6\%\) in the baﬄed cases. For the radial velocity component the peak is slightly underestimated in all bioreactor conﬁgurations by approximately 15-20\% with the highest error being observed at the B2 conﬁguration. These discrepancies could be caused by the fact that ensemble averages were estimated experimentally with a coarser angular resolution (i.e. average is taken considering phase resolved datasets for \(\varphi=0^\circ-55^\circ\) every 5\(^\circ\) thus only 12 phased resolved datasets were used), while LES had a ﬁner angular resolution of 0.5\(^\circ\) (i.e. for \(\varphi=0^\circ-59^\circ\), 119 samples between two consecutive blade passages), which included the entire range of velocity ﬁelds in between two blade passages. Based on the agreement between simulations and experiments and the underestimation of velocities due to the aforementioned limitations of the experimental setup (i.e. coarser angular resolution used for time average calculation), in this chapter the relative error between simulations and experiments was estimated according to \(100 \times \left| \frac{u_{CFD}-u_{PIV}}{u_{CFD}} \right|\) considering that the LES values are representative of the real velocities.

URANS gives a good estimation of the average ﬂow ﬁeld for the baﬄed conﬁgurations (B1 and B2) (Fig. 3.4 and Fig. 3.5). For the vessel with B1 (Fig. 3.4) average vertical proﬁles of \(u_r/u_{tip}\) and \(u_z/u_{tip}\) are in good agreement between URANS and LES at both radial distances \(r/D_T=0.25\) and \(r/D_T=0.35\). For the vessel with B2 (Fig. 3.5) magnitudes of radial velocity
component are satisfactorily estimated by URANS but the location of the maximum $u_r/u_{\text{tip}}$ is predicted at the impeller centreline ($z/D_T=0.35$) whereas for PIV and LES the maximum is located slightly below at $z/D_T=0.33$. This discrepancy is more evident near the impeller region ($r/D_T=0.25$) (Fig. 3.5(a)). In the UB STR the URANS model fails to predict axial and radial velocity distributions both qualitatively and quantitatively (Fig. 3.3). The disagreement between URANS and LES/PIV results can be attributed to the absence of modelling of the free surface. Intense circumferential motion in the UB vessel causes the development of a large vortex on the surface of the liquid which is suppressed by the imposed boundary condition of symmetry at the top of the simulated liquid volume and leads to a distortion of the flow profiles predicted by URANS. Similar results were reported by Ciofalo et al. (1995) who simulated the flow field in a closed UB vessel stirred with a RT and an eight-blade FBT. In their work the use of $k-\varepsilon$ models as a standalone method for flow prediction resulted in erroneous distributions of the individual velocity components due to the rigid-body rotation generated by the absence of baffles. Their results were improved when explicit solution of Reynolds stresses was considered or when $k-\varepsilon$ models were coupled with an additional mathematical model to simulate surface deformation, produced by Nagata et al. (1975) (Nagata, 1975; Ciofalo et al., 1996).

Similarly to the contours of Fig. 3.1, increase of axial and radial velocities with the presence and size of baffles is observed by the vertical profiles of average $u_r/u_{\text{tip}}$ and $u_z/u_{\text{tip}}$ plotted in Fig. 3.3-3.5. For example for $r/D_T=0.25$ and $0.32<z/D_T<0.38$, $u_r/u_{\text{tip}}$ peaks at approximately 0.4 in the UB vessel while it increases ~12% ($u_r=0.45u_{\text{tip}}$) for B1 and ~25% ($u_r=0.5u_{\text{tip}}$) for B2 compared to the UB case. Similar observations could be made when comparing the axial velocity profiles among the different configurations for $r/D_T=0.25$. Above the impeller centreline and at $r/D_T=0.25$ mean $u_z$ is ~1.5 times higher when transitioning from UB to B1 whereas an additional increase of 10% is observed from B1 to B2, confirming the intensification of the upper circulation loop with increasing baffle width, observed from the vector plots of Fig. 3.2.

The impact of baffles on the mean velocity distributions found in this work was compared with the published literature for various reactor configurations. Fan et al. (2021) compared experimental flow data between an unbaffled and a fully baffled (4-baffles) axially agitated vessel and reported ~20% higher velocity magnitude and significant intensification of the axial jet emanating from the impeller in the baffled configuration. They suggested that the impact of baffles on the flow can be associated with the original flow patterns as follows: when
radial impellers are used, radial velocities tend to increase whereas when axial impellers are used axial flow is more enhanced (Fan et al., 2021). Atiben et al. (2013) compared different baffle sizes and geometries in a vessel equipped with a 4-wide blade hydrofoil and discussed that the increase in baffle size amplified the drag of the system and enhanced the individual circulation loops (Atiben and Gao, 2013). Dong et al. (2016) examined the impact of baffle length on the flow patterns in radially agitated flat and dished bottom tanks at Re~14,000. They found that the presence of shorter baffles led to the formation of a large dead zone below the impeller which was more pronounced in the dished bottom tank. The extension of the baffle length up to the bottom of the tank reduced the volume of the dead zone by increasing the velocity magnitude and enhancing the circulation loops (Jie Dong et al., 2016).

Average velocities were compared to previously published results for all reactor configurations studied, including baffled and unbaffled (Escudié and Liné, 2003; Hartmann et al., 2004; Alcamo et al., 2005; Delafosse et al., 2009; Zhao, Gao and Bao, 2011; Gimbun et al., 2012). It should be noted that the impeller geometry used in this work is different from impeller configurations extensively examined in the past, such as the commonly used RT (Escudié and Liné, 2003; Alcamo et al., 2005; Gimbun et al., 2012) or four-blade FBT (Steiros et al., 2016, 2017a). For an unbaffled configuration as in this work, Alcamo et al. investigated the flow produced by a RT for Re=30,000 and found maximum values of radial component, \(u_r/u_{tip}=0.22\) lower than those found in the current work (Alcamo et al., 2005). The work of Delafosse et al. (2008) and Escudié et al. (2003) investigated the flow in a baffled radially agitated tank with a RT both computationally and experimentally (Re~150,000) and reported maximum \(u_r\sim0.7u_{tip}\), approximately 40% higher than the maximum \(u_r/u_{tip}\) found in the present work for the baffled case with B2 (Escudié and Liné, 2003; Delafosse et al., 2008). Likewise, Hartmann et al. (2004) used LES and URANS simulations to investigate the flow field in a similar tank at Re~7,300 and found maximum \(u_r\sim0.65u_{tip}\) in proximity to the impeller tip (versus \(u_r\sim0.5u_{tip}\) which is the predicted mean radial velocity in the current work for the baffled vessel with B2). At a smaller scale, Collignon et al. (2016) conducted LES to investigate the flow field produced by an assortment of axial and radial impellers in a 200 mL STR equipped with two probes. They discussed that the presence of two probes might have an impact on the flow equivalent to baffles and reported that for the case of a RT contours of velocity magnitude at Re=2,613 peaked at \(|u|\sim0.7u_{tip}\), which is similar to the results presented in Fig. 3.1 for the baffled configurations (Collignon et al., 2016). In the aforementioned studies, LES satisfactorily reproduced the generated velocity field and were in agreement with
experimental data in both un baffled and baffled bioreactors (Hartmann et al., 2004; Alcamo et al., 2005; Delafosse et al., 2008). Differences related to impeller design, reactor volumes (250 mL in the current work versus 3-70 L in the cited literature for standard reactor geometries) and flow regime (Re=3,732 in the current work versus Re~10,000-40,000 in the cited literature) must be considered when comparing the current and other configurations and highlights the demand of a thorough characterisation for both baffled and un baffled configurations of small volume STRs.

3.2.1.2 Impact of baffles on tangential fluid motion and simulation of free surface

The impact of baffles on the fluid circumferential motion was examined by measuring the average tangential velocity component $u_\theta / u_{tip}$ for the three bioreactor configurations (UB, B1 and B2) and results are presented in Fig. 3.6. In Fig. 3.6(a) vertical profiles of the mean $u_\theta / u_{tip}$ are presented at $r/D_T=0.25$ extracted from LES and URANS simulations. Ensemble average $u_\theta / u_{tip}$ increases at $0.3<z/D_T<0.4$ and peaks for all configurations at the impeller centreline. Peak values of tangential velocity were influenced by the presence and the size of baffles with slightly higher values observed for the UB case ($u_{\theta,max} \sim 0.6u_{tip}$, $u_{\theta,max} \sim 0.55u_{tip}$ and $u_{\theta,max} \sim 0.5u_{tip}$ for UB, B1 and B2, respectively). Apart from the differences observed around the impeller area, vertical profiles of mean $u_\theta / u_{tip}$ deviate largely in the regions above $0.4<z/D_T<0.8$ and below $0<z/D_T<0.3$ the impeller. Tangential velocities are expected to maximise in the impeller region, where fluid is discharged from the impeller blades, and get significantly lower in regions away from the impeller stream. In these areas mean $u_\theta / u_{tip}$ values extracted by LES decrease two-fold when transitioning from the UB to the baffled vessel with B1 while increase of the baffle size (B2) resulted to a further 50% $u_\theta / u_{tip}$ reduction confirming that the intensity of circumferential motion is a function of baffle presence but also strongly depends on baffle size. For example, at $0.4<z/D_T<0.9$, $u_\theta \sim 0.4u_{tip}$ for the unbaffled case and is reduced to $0.2u_{tip}$ and $0.1u_{tip}$ with the presence of B1 and B2 respectively.

The tangential velocity field was not adequately reproduced by URANS simulations in the UB tanks. Even though the model is in agreement with LES with respect to $u_\theta$ peak value at the impeller centreline, in the areas above and below the impeller mean $u_\theta / u_{tip}$ is predicted ~30% higher than in the LES. URANS prediction was improved in baffled configurations where the relative error $100 \times \left| \frac{u_\theta,LES-u_\theta,RANS}{u_\theta,LES} \right|$ was reduced to ~20% for B1 while for B2 results obtained from LES and URANS were in excellent agreement. The overestimation of $u_\theta / u_{tip}$
away from the impeller region by URANS can be attributed to the suppression of the surface vortex due to the imposed symmetry boundary condition at the top of the vessel. The error is higher in the UB reactor where the depth of the surface vortex is increased impacting also the model predictions for average $u_r/utip$ and $u_z/utip$ as discussed in Section 3.2.1.1 and presented in Fig. 3.3. The impact of baffles on tangential velocity was also discussed by Ammar et al. (2011) who used the RANS approach to model the influence of baffle presence and length on a closed vessel equipped with a RT, with MRF. They found that in an UB vessel tangential velocities where $\sim0.4utip$ above the impeller, similar values with those reported in the current work, while model prediction improved significantly after the addition of baffles (Ammar et al., 2011). The above underline the sensitivity of RANS models to accurately predict the flow patterns in closed UB bioreactors in the absence of a model accounting for the deformation of the free surface and highlights the reliability of LES especially for modelling the flow around the impeller area (Yang and Zhou, 2015; de Lamotte et al., 2018b).

Average tangential velocity was experimentally validated for the baffled configuration with B1. Velocities were measured at horizontal planes at different tank elevations $0.15<z/D_T<0.56$ and results are presented in Fig. 3.6(b). At the impeller centreline ($z/D_T=0.35$) PIV and LES results are in good agreement. In proximity to the impeller tip ($r/D_T\sim0.23$) mean $u_\theta/utip \sim0.8$ while it abruptly decays as the radial distance increases. At $z/D_T=0.56$ and $z/D_T=0.15$, the error between PIV and LES is 10-25% with higher error existing in the region below the impeller. Above the impeller average $u_\theta/utip \sim0.2$, thus confirming the existence of circumferential motion predicted by LES (Fig 3.6(a) for B1). Experimental and computational measurements are in a better agreement in the areas closer to the impeller tip indicating that tangential velocity estimation away from the impeller swept volume is affected by the suppression of the free surface even when LES was used. Nevertheless, those discrepancies between LES and PIV do not impact the model predictions for axial and radial velocity components in none of the vessels studied (UB, B1 and B2) (Fig. 3.3-Fig. 3.5). Those results confirm the importance of baffles in the decrease of solid body rotation and highlight the impact of baffle size on its reduction and CFD simulation.

**3.2.1.3 Phase resolved velocity field distribution**

Vertical profiles of the phase resolved radial and axial velocity components, $\langle u_r \rangle/utip$ and $\langle u_z \rangle/utip$, are presented for different impeller phase angles ($\varphi=10^\circ$, $30^\circ$ and $50^\circ$) and for radial positions in proximity to the trailing vortices for all the bioreactor types examined (UB, B1 and B2) in Fig. 3.8, Fig. 3.10 and Fig. 3.12. Validation for the phase resolved velocity field
is only presented for the LES as with the URANS approach only the average flow field is
resolved and evolution of the periodic motion (i.e. trailing vortices) is not adequately
accounted for (Aubin, Fletcher and Xuereb, 2004b; Delafosse et al., 2008). Phase resolved vector
fields for each configuration are presented in Fig. 3.7, Fig. 3.9 and Fig. 3.11 and approximate
location of the trailing vortices, where vertical $\langle u_r \rangle / u_{tip}$ and $\langle u_z \rangle / u_{tip}$ are plotted, are indicated
with the red arrow. For the UB bioreactor, vertical profiles of axial and radial velocity
components are illustrated at $r/D_T = 0.22$ for $\phi = 10^\circ$, $r/D_T = 0.25$ at for $\phi = 30^\circ$ and $r/D_T = 0.27$
for $\phi = 50^\circ$, in Fig. 3.8. For the baffled vessels vertical profiles of $\langle u_r \rangle / u_{tip}$ and $\langle u_z \rangle / u_{tip}$ are
illustrated at $r/D_T = 0.23$ for $\phi = 10^\circ$, $r/D_T = 0.26$ for $\phi = 30^\circ$ and $r/D_T = 0.30$ for $\phi = 50^\circ$, in Fig.
3.10 for B1 and at $r/D_T = 0.23$ for $\phi = 10^\circ$, $r/D_T = 0.27$ for $\phi = 30^\circ$ and $r/D_T = 0.31$ for $\phi = 50^\circ$, in
Fig. 3.12 for B2. In all vessels studied, vertical profiles of $\langle u_r \rangle / u_{tip}$ show that radial velocity
reaches a maximum at $10^\circ$ behind the blade, when the trailing vortices have fully formed. As
the blade progresses and the intensity of the trailing vortices weakens, the radial velocity,$\langle u_r \rangle / u_{tip}$, gets progressively smaller as well. Overall, vertical profiles of $\langle u_r \rangle / u_{tip}$ and
$\langle u_z \rangle / u_{tip}$ indicate very good agreement between the PIV and LES phase resolved estimates. In
the baffled vessel with B2 velocity profiles are slightly underestimated by the experimental
measurements with an average error ~15%. This can be attributed to the fact the camera used
was not fast enough to capture increases in velocity magnitude and fluctuations without
compromising the quality of the images.

Consistent with previous observations reported in Section 3.2.1.1 for the average velocity
field, maximum radial velocities increase in the baffled tanks and with increasing baffle size.
For example, for the UB case at $\phi = 10^\circ$ maximum $\langle u_r \rangle \sim 0.55 u_{tip}$ whereas for the same phase
angle in the baffled vessels, $\langle u_r \rangle$ peaks at $\sim 0.62 u_{tip}$ and $\sim 0.65 u_{tip}$ for B1 and B2 respectively.
Similarly, axial velocity profiles for the baffled vessels in Fig. 3.10 and Fig. 3.12 appeared
broader and obtained higher values compared to the UB case of Fig. 3.8 (i.e. $\langle u_z \rangle \sim \pm 0.2 u_{tip}$ in
B2 versus $\pm 0.15 u_{tip}$ in the UB vessel for $10^\circ < \phi < 30^\circ$). The trailing vortices were identified and
are illustrated on the phase resolved vector fields presented in Fig. 3.7, Fig. 3.9 and Fig. 3.11
for UB, B1 and B2 reactors, respectively. Although, the trailing vortices are more intense at
$\phi = 10^\circ$ and move further out in the radial direction as the phase angle increases, in the UB
vessel they appeared more distinctively. However, at $\phi \geq 30^\circ$ an inclination of the impeller jet
is evident compromising their stability in the baffled vessels. This phenomenon is related to
trailing vortex stability and will be discussed in detail in chapter 4.
Gimbun et al. (2012) used RANS and subgrid scale models (including DES and LES) to fully characterise ensemble and phase resolved velocity fields in a baffled STR with a RT at \( Re \sim 29,000 \). They reported maximum radial velocities ranging between \( \langle u_r \rangle \sim 0.6 u_{tip} \) for \( \varphi = 0^\circ - 59^\circ \) at radial positions close to the tip of the blade and \( \langle u_r \rangle \sim 0.5 u_{tip} \) for \( \varphi = 0^\circ - 59^\circ \) further away from the impeller (Gimbun et al., 2012). Steiros et al. (2017) used a four-blade FBT to experimentally study the velocity field in a prismatic-octagonal tank at \( Re \sim 150,000 \) and reported \( 0.2 u_{tip} < u_r < 0.65 u_{tip} \) for \( \varphi = 30^\circ - 60^\circ \) (Steiros, 2017; Steiros et al., 2017). As discussed before, discrepancies between published data and values obtained in the current work, may be attributed to different bioreactor and impeller geometries, working volumes and Reynolds number.

### 3.2.2 Impact of baffles on turbulence distribution

Turbulence is an indication of velocity fluctuations in STRs and its accurate evaluation is challenging both experimentally and computationally due to the necessity of very fine spatial and temporal resolution in the 3D flow domain (Escudié and Liné, 2003). Several works have focused on the characterisation on turbulence in STRs in baffled (Escudié and Liné, 2003; Ducci and Yianneskis, 2005b; Delafosse et al., 2009; Collignon et al., 2016) and UB (Alcamo et al., 2005; Steiros et al., 2017a) vessels. Estimation and validation of turbulence levels is important as it would give an indication of shear levels and mixing efficiency in the interior of the vessels (Odeleye et al., 2014). The bioreactor used in this work was designed and used for mammalian cell processing, therefore characterisation of turbulence and shear are essential. Turbulence measures velocity fluctuations thus it is expected that regions of high turbulence in STRs will be translated to regions of increased shear levels.

#### 3.2.2.1 Periodic kinetic energy distribution

Contribution of the periodic motion to the kinetic energy was calculated across all the bioreactors studied for both experiments and simulations. Vertical profiles of averaged periodic kinetic energy \( k_{per} \) are presented in Fig. 3.13 in proximity to the impeller tip at \( r/D_T = 0.25 \) where the intensity of the organised motion is expected to be high. LES results are overall in good agreement with the experimental data for the UB and baffled configurations studied. Discrepancies between experiments and simulations become slightly higher in the baffled vessels where the relative error \( (100 \times \frac{k_{per,CFD} - k_{per,PIV}}{k_{per,CFD}}) \) is estimated \( \sim 15\% \) and \( \sim 20\% \) for B1 and B2 configurations, respectively, with LES results giving higher values. As discussed in Section 3.2.1.1 for the average velocity field, ensemble averaged periodic kinetic energy was
calculated experimentally considering 12 phase resolved datasets (0-55° with a step of 5°) as opposed to LES where 119 samples were considered, each one taken every 0.5° between two consecutive blade passages. Higher resolution in LES provided access to almost the entire range of velocity field between two blade passages capturing turbulence in a better way.

In URANS simulations, since the instantaneous flow field is not directly computed, the periodic kinetic energy was estimated by assuming the model resolved a phase resolved velocity field (Delafosse et al., 2008) and was found to be consistently lower than the one predicted by LES or measured by PIV. Such underestimation can be attributed to the fact that the velocity decomposition is limited to the averaged velocity data extracted for each time step and the mean velocity. Therefore the periodicity due to trailing vortex formation at different phase angles \( \varphi \) is not adequately accounted for.

The magnitude of periodic kinetic energy increases with baffle presence and size. In the UB case \( k_{\text{per}}/u_{\text{tip}}^2 \) is estimated approximately 45% lower than in the baffled configuration with B1 while transition from B1 to B2 leads to an additional increase of \( \sim45\% \) for the latter. Validation outcomes for the average \( k_{\text{per}}/u_{\text{tip}}^2 \) are in agreement with work published by Delafosse et al. (2008) who used LES and k-\( \varepsilon \) Standard (URANS) models to simulate the flow in a radially agitated vessel with a working volume of \( \sim70 \text{ L} \) and \( Re \sim 150,000 \). In their work it was mentioned that URANS failed to reproduce the average \( k_{\text{per}} \) as mean values were approximately three times lower than those estimated from LES and PIV \( (k_{\text{per}} \sim 0.03u_{\text{tip}}^2 \text{ based on URANS versus } k_{\text{per}} \sim 0.1u_{\text{tip}}^2 \text{ based on LES}) \) (Delafosse et al., 2008).

### 3.2.2.2 Turbulent kinetic energy distribution

A direct comparison between turbulent characteristics of the three configurations, UB, B1 and B2, is provided in Fig. 3.14 where in-plane \( k' \), extracted from LES and validated via PIV, are presented. The ensemble averaged turbulent kinetic energy was obtained by averaging the corresponding phase resolved values with 0.5° and 5° resolution for the LES and PIV respectively. Results of Fig. 3.14 correspond to ensemble average distribution of \( k'/u_{\text{tip}}^2 \) calculated based on eq. 2.42-2.43. With respect to the experimental validation of the average \( k' \) predicted by LES, the agreement is good with approximately an error \( (100 \times \frac{k'_{\text{CFD}}-k'_{\text{PIV}}}{k'_{\text{CFD}}}) \) of 10% between PIV and LES with the former slightly underestimating the maximum values predicted by the model. Similarly to the average flow field discussed in Section 3.2.1.1, discrepancies can be attributed to the fact that ensemble averaged turbulent kinetic energy was calculated experimentally with a coarser temporal resolution than LES.
Vertical profiles of $k'/u_{tip}^2$, are illustrated in Fig. 3.15, near the impeller tip. Turbulent kinetic energy peaks at $0.25<r/D_T<0.27$ depending on the configuration used, with $r/D_T=0.26$, $r/D_T=0.25$ and $r/D_T=0.27$ being the radial positions of maximum $k'$ for the UB, B1 and B2 configurations, respectively. Vertical profiles for CFD and PIV are presented for the different configurations in Fig. 3.15(a) in close proximity to the impeller tip, at $r/D_T=0.25$, following the isotropic turbulence assumption (2D calculation based on the fluctuating radial and axial velocity components $u'_r$ and $u'_z$, respectively for the vertical plane of measurement) according to eq. 2.43. For B1 configuration the overall distribution of $k'$ is satisfactorily predicted by both URANS and LES and the mean values at this radial distance ($r/D_T=0.25$) are similar between experiments and simulations (Fig. 3.15(a)). For the B2 configuration, URANS estimated a ~20% lower peak value for $k'/u_{tip}^2$ and predicted its position on the impeller centreline ($z/D_T=0.35$) as opposed to LES and PIV which showed that the area of maximum $k'/u_{tip}^2$ occurs at $z/D_T=0.33$ (similar to what was observed for radial velocity calculated by URANS in B2 vessel in Fig. 3.5). In the UB configuration, while PIV and LES are in a good agreement, URANS seems to misestimate both the values and distribution of $k'/u_{tip}^2$. Such underprediction by URANS is expected based on previous findings for the velocity field described in Sections 3.2.1.1 and 3.2.1.2 and is attributed to the absence of the simulation of the free surface.

Turbulent kinetic energy is found to increase three-fold with the addition of baffles and with baffle size. For example in the UB vessel average $k'/u_{tip}^2$~0.05 and increases to $k'/u_{tip}^2$~0.1 and $k'/u_{tip}^2$~0.15 for the configurations with B1 and B2, respectively. The higher levels of $k'$ observed in the baffled tanks can be attributed to the increased drag imposed by the presence of baffles which enhances the flow resistance (Atibeni and Gao, 2013). This also leads to a ~2.5 times higher $P_0$ (as it will be discussed in chapter 5), which is subsequently associated with similar increase in energy dissipation rate ($\varepsilon$) as $P_0$ and ($\varepsilon$) are directly proportional (eq. 2.4). Specifically, volume average $\varepsilon$ was extracted by the URANS simulation and was estimated ~$0.2N^3D_i^2$ and 0.5$N^3D_i^2$ for the UB and B2 configurations respectively (corresponding to 2.5 times increase).

The impact of baffles on turbulence distribution has been discussed by Fan et al. (2021) based on PIV results in axially agitated vessels with and without baffles. They reported that the axial and radial root mean square (rms) components of the flow appeared higher in the presence of baffles, enhancing the overall turbulent levels (Fan et al., 2021). Similarly, Tamburini et al. (2019), used k-ω RANS models for radially baffled and UB tanks to estimate $P_0$ in a broad range.
of $Re$, covering the transitional and fully turbulent regime ($Re\sim600$-$33,000$) and reported that $P_0$ increased in the presence of baffles suggesting that turbulence levels will also increase (Tamburini et al., 2019).

The 2D isotropic assumption for turbulence (based on eq. 2.43) was compared with the resulting turbulent kinetic energy calculated from fluctuating velocity components in all three directions, $u'_r$, $u'_z$ and $u'_\theta$, according to eq. 2.40 (Fig. 3.15(b)). For this purpose LES data was used, as the instantaneous velocity field is resolved for each velocity component across the entire range of impeller positions. The maximum difference between the predicted 2D and 3D turbulent kinetic energy is observed for the UB vessel where the latter peaked at a value $\sim$35% lower in comparison to values predicted using the isotropic assumption. The discrepancies between 2D and 3D turbulence calculation are reduced to $\sim$20 and $\sim$15% in the presence of baffles B1 and B2, respectively. Qualitative representation of the fluctuations in the $\theta$ direction predicted under the assumption of isotropic turbulence ($0.5(u'_r^2 + u'_z^2)/u_{tip}^2$) and those estimated from the tangential fluctuating velocity component via LES ($u'_\theta^2/u_{tip}^2$), is presented in the contours of Fig. 3.16(a) and Fig. 3.16(b) respectively for all configurations. The $0.5(u'_r^2 + u'_z^2)/u_{tip}^2$ component is always overestimating the actual velocity fluctuations in $\theta$ direction, $u'_\theta^2/u_{tip}^2$, regardless of the presence and size of baffles with the higher discrepancies corresponding to the unbaffled tank. For example, in the UB case the $0.5(u'_r^2 + u'_z^2)/u_{tip}^2$ is approximately 65% higher than the corresponding $u'_\theta^2/u_{tip}^2$ as opposed to $\sim$30% and $\sim$20% overestimation of $0.5(u'_r^2 + u'_z^2)/u_{tip}^2$ over $u'_\theta^2/u_{tip}^2$ for the baffled vessels with B1 and B2 respectively. The lower values of $u'_\theta^2/u_{tip}^2$ can explain the discrepancies between 2D and 3D estimation of turbulent kinetic energy presented in Fig. 3.15 and confirm the overprediction when isotropic assumption is implemented. The development of high solid body rotation in the UB tank increases significantly the tangential velocity component leading to undisturbed circumferential motion with low fluctuations which can justify the low values of $u'_\theta^2/u_{tip}^2$ found. The addition of baffles disrupts the solid body rotation and increases the resistance of the system leading to an increase in the fluctuating tangential velocity component which is translated to the higher turbulence levels identified in B1 and B2 cases.

Turbulent kinetic energy prediction by both approaches indicated that 3D calculation leads to a smoother and clearer representation of the characteristic double peak at $0.32<z/D_T<0.38$ corresponding to the impeller trailing vortices. Moreover, the double peak in average $k'/u_{tip}^2$ is not adequately predicted by the PIV results which could be attributed to the coarser
temporal resolution used to obtain the ensemble averaged measurements (described in Section 3.2.1.1). The double peak is also absent from URANS results but this could be because of the averaging approach of those models which doesn’t account for thorough trailing vortex analysis but also can be due to modelling the velocity fluctuations (Delafosse et al., 2008, 2009). Similar results were reported by Khan et al. (2006) who compared 3D and 2D turbulence calculations in a baffled vessel equipped with a PBT. They reported that the tangential velocity component was satisfactorily predicted by the isotropic assumption suggesting an overall agreement between the two calculations with small discrepancies with respect to the location and intensity of the maximum turbulent kinetic energy obtained at the vortex core which was better estimated by the 3D estimation of turbulence (Khan, Rielly and Brown, 2006). In the same context Kresta et al. (1996) carried out a similar comparison for baffled radially and axially agitated vessels based on 3D LDA data and used a single velocity component for the estimation of $k'$. They concluded that depending on the flow type promoted by each impeller the main flow component can be used to satisfactorily predict $k'$: in applications with axial impellers the $u'_z$ component, and in applications with radial impellers the $u'_r$ component can be solely used to estimate $k'$ in an acceptable way (Zhou and Kresta, 1996).

Average values of turbulent kinetic energy were compared to previously published results for both baffled and UB vessels (Hartmann et al., 2004; Alcamo et al., 2005; Delafosse et al., 2009; Zhao, Gao and Bao, 2011; Gim bun et al., 2012). For an unbaffled configuration as in this work, Alcamo et al. investigated the flow produced by a RT for $Re=30,000$ and found maximum values of turbulent kinetic energy $k'/u^2_{tip}=0.05$ close to those found in the current work (Alcamo et al., 2005). The work of Steiros et al. with an octagonal unbaffled tank and a four blade FBT was denoted by higher levels of turbulence intensity $(\sqrt{\langle u^2_z \rangle + \langle u^2_z \rangle}/u_{tip} \sim 0.45$ versus 0.24 in the current work), as the corner of the prism wall might have resulted in a baffle effect (Stei ros et al., 2017a). When baffled tanks were considered, Lee et al. reported higher values for $k'$ than those found in the current work $(k'/u^2_{tip} = 0.15$ versus 0.2) (Lee and Yianneskis, 1998). As discussed previously, differences related to impeller design, reactor volumes and flow regime must be considered when comparing the current with other configurations and underlines the need of a thorough characterisation for both baffled and unbaffled configurations of small volume STRs.

Contours of the average $k'/u^2_{tip}$ (Fig. 3.14) indicated that turbulent kinetic energy develops, at the interface between the trailing vortices, in agreement with (Liu et al., 2010). Vertical profiles of the average $k'$ at $0.32<r/D_T<0.38$ showed that $k'/u^2_{tip}$ is up to three times higher than the
average periodic turbulent component. For example in the UB case \( k_{\text{per, max}} \sim 0.025u_{\text{tip}}^2 \) and \( k' \sim 0.06u_{\text{tip}}^2 \) whereas in the baffled bioreactors \( k_{\text{per, max}} \sim 0.035u_{\text{tip}}^2 \) and \( k' \sim 0.1u_{\text{tip}}^2 \) for B1 and \( k_{\text{per, max}} \sim 0.055u_{\text{tip}}^2 \) \( k' \sim 0.15u_{\text{tip}}^2 \) for B2 (Fig. 3.13 and Fig. 3.15). Escudié et al. (2003) mentioned that \( k_{\text{per}}/u_{\text{tip}}^2 \) is expected to be higher than \( k'/u_{\text{tip}}^2 \) close to the impeller blades where the flow is highly dependent on the blade passage and trailing vortex formation, and energy transfer occurs between the organised and the mean motion. As the radial distance from the tip of the blade increases, \( k_{\text{per}}/u_{\text{tip}}^2 \) becomes minimum and acts as a source of turbulence that will make \( k'/u_{\text{tip}}^2 \) to increase (source and sink relationship) (Escudié and Liné, 2003). Variations in periodic kinetic energy are also observed among published data. For example according to work conducted by Liu et al. (2010), who examined the turbulence levels in a baffled tank equipped with a deep-hollow blade disk turbine operating at \( Re \sim 9,000 \), the average \( k_{\text{per}}/u_{\text{tip}}^2 \sim 0.03 \) was 2.6 times lower than \( k'/u_{\text{tip}}^2 \) and approximately 3 times lower than the values reported by Delafosse et al. (2008) and Escudié et al. (2003) (Escudié and Liné, 2003; Delafosse et al., 2008; Liu et al., 2010). In the current work (\( Re \sim 3,732 \)) the maximum for the average \( k_{\text{per}} \sim 0.035u_{\text{tip}}^2 \) and \( 0.055u_{\text{tip}}^2 \) for the baffled configurations with B1 and B2 respectively, while it is expected to increase at least three-fold in different phase angles \( \varphi \) when the trailing vortices are evolving. The above data show that when periodic kinetic energy across different bioreactors is assessed (either results of the current work versus published literature or results across different published works) variations with respect to the vessel geometry, impeller type and \( Re \) should be considered as those may lead to highly deviating values. The lower levels of periodic turbulence found in the current work imply lower periodic stability of the trailing vortices which could be translated to an increase in the fluctuating component.

### 3.3 Summary

The objective of the work presented in this chapter was to assess the impact of baffles and baffle size on the mean flow field in a scale-down model, and evaluate the performance of CFD modelling when compared with experimental PIV data. The mean flow patterns were examined for three bioreactor configurations including one un baffled and two baffled configurations with increasing baffle size (B1<B2). The impact of baffles on hydrodynamics was explored based on velocity and turbulence profiles. The transition from UB to baffled configurations resulted in higher radial and axial velocities due to the abrupt reduction of tangential velocity when baffles are present. Turbulence levels were also amplified three times as baffles increase the resistance of the system, resulting in an increase in Power number.
For the computational characterisation of the flow field two CFD models were implemented: k-ε Realizable model based on RANS approach and LES, both operating with the SM method. LES was in good agreement with experimental PIV data for both phase and time resolved velocity fields. RANS modelling led to satisfactory predictions of the distributions of average radial and axial velocities but for the unbaffled case the model failed to reproduce the average flow field.

A study for the prediction of the average tangential velocities across the different models was carried out and showed that maximum values were acceptably predicted by both RANS and LES across all the configurations studied. Large variations were observed in the areas above and below the impeller region where RANS overestimated LES predictions by approximately 30% for the UB case. Such overprediction of tangential velocity component by RANS implies the sensitivity of the model to accurately predict the flow patterns in a closed UB vessel in the absence of a model in which the deformation of the free surface will be considered, and accounts for the misestimation of the radial and axial velocity distributions. Nevertheless, in these regions high tangential velocities were also evident for LES for both UB and baffled vessels with B1, indicating that the intensity of the circumferential motion and solid body rotation is a function of both baffle presence and size.

Periodic and turbulent kinetic energy were also estimated and validated with PIV. LES and PIV were in good agreement for both turbulent components across all vessel configurations examined. Periodic kinetic energy was severely underpredicted by URANS but this can be attributed to the fact that these models only resolve the average flow field, thus the accurate prediction of the periodic motion and trailing vortices is not accounted for. Both turbulent components increased with presence and size of baffles with $k_{\text{per}}$ appearing 40-50% higher for each baffle addition (B1 and B2) and $k'$ been measured three times higher between UB and B2 vessels. Estimations of turbulent kinetic energy based on isotropic 2D and 3D assumptions showed satisfactory agreement between experiments and simulations with the latter having slightly lower magnitude. Evaluation of the turbulent fluctuations in the $\theta$ direction was conducted based on the LES dataset and showed that the tangential fluctuating velocity component was always overestimated by the 2D prediction. Nevertheless, profiles of turbulent kinetic energy distribution considering all the velocity components led to better qualitative representation of the characteristic double peak corresponding to trailing vortex formation.
Velocity profiles and turbulence levels were assessed and compared with data available in the published literature for standard reactor designs. Variations with respect to velocity data were low between the current and published works but turbulence levels reported here are lower, implying that differences in the geometry and operation among different vessels should always be taken into account. Nevertheless, experimental validation of LES across both UB and baffled configurations studied in this work, highlights the reliability of the model at intermediate $Re$, where SDMs tend to operate. Thereinafter, the validated LES data are used to further analyse the flow structures focusing on the investigation of formation and stability of trailing vortices emanating from the FBT across the different configurations studied.
Figure 3.1: Impact of baffle size on velocity magnitude ($|u|/u_{tip}$) for (a) LES and (b) PIV. For baffled vessels the vertical plane is $P_1$. Plots for the EA velocity magnitude in the UB case are presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 3.2: Impact of baffle size on the intensity of the impeller circulation loops. Vector plots are presented for (a) UB and baffled configurations with (b) B1 and (c) B2. Results are based on LES dataset for the vertical profiles of UB and baffled (B1, B2) vessels for plane P₁.
Figure 3.3: Vertical profiles of ensemble average $u_r/u_{tip}$ and $u_z/u_{tip}$ for the UB configuration (schematic presented on the right hand side) for two different radial distances: (a) $r/D_T=0.25$ and (b) $r/D_T=0.35$. The solid horizontal line in all figures represents the impeller centreline.
Figure 3.4: Vertical profiles of ensemble average $u_r/u_{tip}$ and $u_z/u_{tip}$ for the baffled configuration with B1 at P1 for two different radial distances: (a) $r/D_T=0.25$ and (b) $r/D_T=0.35$. The solid horizontal line in all figures represents the impeller centreline.
Figure 3.5: Vertical profiles of ensemble average $u_r/u_{tip}$ and $u_z/u_{tip}$ for the baffled configuration with B2 at P$_1$ for two different radial distances: (a) $r/D_T=0.25$ and (b) $r/D_T=0.35$. The solid horizontal line in all figures represents the impeller centreline.
Figure 3.6: Ensemble average $u_\theta/u_{tip}$: (a) vertical profiles obtained from URANS (dashed lines) and LES (continuous lines) for UB (blue), B1 (black) and B2 (grey) configurations at $r/D_T=0.25$, (b) experimental validation for B1 configuration. Average $u_\theta/u_{tip}$ was measured at different tank elevations ($z/D_T=0.56$, $z/D_T=0.35$ and $z/D_T=0.15$) to show impact of baffle size on fluid circumferential motion away from the level of impeller. Vertical profiles based on CFD are presented for plane $P_1$. 
Figure 3.7: Phase resolved velocity fields for UB configuration (schematic presented on the right hand side) for (a) PIV and (b) LES for $\varphi=10^\circ$, $\varphi=30^\circ$ and $\varphi=50^\circ$. Red arrows indicate an approximate location of the trailing vortex core which is the position used to plot the vertical profiles of axial and radial velocities in Fig. 3.8 ($r/D_T = 0.22$ for $\varphi=10^\circ$, $r/D_T = 0.25$ for $\varphi=30^\circ$, $r/D_T = 0.27$ for $\varphi=50^\circ$).
Figure 3.8: Vertical profile of $(u_r)/u_{tip}$ ((a), (c) and (e)) and $(u_z)/u_{tip}$ (b, d and f) for $\phi=10^\circ$ ((a) and (b)), $\phi=30^\circ$((c) and (d)) and $\phi=50^\circ$ ((e) and (f)) behind the impeller blade. Velocities are validated close to the vortex core for each angle ($r/D_T=0.22$ for $\phi=10^\circ$, $r/D_T=0.25$ for $\phi=30^\circ$, $r/D_T=0.27$ for $\phi=50^\circ$). Results correspond to the UB configuration (schematic presented on the right hand side). The solid horizontal line in all figures represents the impeller centreline. Plots for the phase resolved axial and radial velocities in the UB configuration are presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 3.9: Phase resolved velocity fields for B1 configuration at P₁ for (a) PIV and (b) LES for ϕ=10°, ϕ=30° and ϕ=50°. Red arrows indicate an approximate location of the trailing vortex core which is the position used to plot the vertical profiles of axial and radial velocities in Fig. 3.10 (r/Dₜ~0.23 for ϕ=10°, r/Dₜ~0.26 for ϕ=30°, r/Dₜ~0.3 for ϕ=50°).
Figure 3.10: Vertical profile of $\langle u_r \rangle / u_{tip}$ ((a), (c) and (e)) and $\langle u_z \rangle / u_{tip}$ ((b), (d) and (f)) for $\varphi=10^\circ$ ((a) and (b)), $\varphi=30^\circ$ ((c) and (d)) and $\varphi=50^\circ$ ((e) and (f)) behind the impeller blade. Velocities are validated close to the vortex core for each angle ($r/D_T \sim 0.23$ for $\varphi=10^\circ$, $r/D_T \sim 0.26$ for $\varphi=30^\circ$, $r/D_T \sim 0.3$ for $\varphi=30^\circ$). Results correspond to B1 configuration at $P_1$. The solid horizontal line in all figures represents the impeller centreline.
Figure 3.11: Phase resolved velocity fields for B2 configuration at P₁ for (a) PIV and (b) LES for $\varphi=10^\circ$, $\varphi=30^\circ$ and $\varphi=50^\circ$. Red arrows indicate an approximate location of the trailing vortex core which is the position used to plot the vertical profiles of axial and radial velocities in Fig. 3.12 ($r/D_T\sim0.23$ for $\varphi=10^\circ$, $r/D_T\sim0.27$ for $\varphi=30^\circ$, $r/D_T\sim0.31$ for $\varphi=50^\circ$).
Figure 3.12: Vertical profile of $\langle u_x \rangle / u_{tip}$ ((a), (c) and (e)) and $\langle u_z \rangle / u_{tip}$ ((b), (d) and (f)) for $\varphi = 10^\circ$ ((a) and (b)), $\varphi = 30^\circ$ ((c) and (d)) and $\varphi = 50^\circ$ ((e) and (f)) behind the impeller blade. Velocities are validated close to the vortex core for each angle ($r/D_T \approx 0.23$ for $\varphi = 10^\circ$, $r/D_T \approx 0.27$ for $\varphi = 30^\circ$, $r/D_T \approx 0.31$ for $\varphi = 30^\circ$). Results correspond to B2 configuration at $P_1$. The solid horizontal line in all figures represents the impeller centreline.
Figure 3.13: Vertical profiles of periodic kinetic energy ($k_{per}/u_{tip}^2$) presented for PIV and LES for all three configurations studied at $r/D_T=0.25$: (a) UB, (b) B1 and (c) B2. For baffled vessels the vertical plane is $P_1$. The solid horizontal line in all figures represents the impeller centreline.
Figure 3.14: Impact of baffle size on turbulent kinetic energy ($k'/u_{tip}^2$) for (a) LES and (b) PIV. For baffled vessels the vertical plane is P₁.
Figure 3.15: Vertical profiles of turbulent kinetic energy ($k'/u_{tip}^2$) presented for PIV and LES for all three configurations studied (UB, B1, B2) at $r/D_T=0.25$: (a) $k'/u_{tip}^2$ presented based on the isotropic turbulence assumption for LES (LES 2D), URANS and PIV and (b) 2D calculation of $k'$ compared with 3D estimation based on LES results (LES 3D). For baffled vessels the vertical plane is $P_1$. The solid horizontal line in all figures represents the impeller centreline.
Figure 3.16: Turbulence fluctuations in \( \theta \) direction based on (a) isotropic turbulence assumption, \( 0.5(u_r'^2 + u_z'^2)/u_{tip}^2 \), and (b) estimation of \( u_{\theta}'^2/u_{tip}^2 \) based on LES results across the three vessel configurations studied (UB, B1 and B2). For baffled vessels the vertical plane is \( P_1 \).
Chapter 4: Impact of baffles on trailing vortex formation and flow instabilities

4.1 Introduction

The aim of this chapter is to study the impact of baffle presence, size and impeller disk on the stability of the trailing vortices generated by a radial impeller (FBT, RT). Trailing vortices characterise the periodic flow patterns, determine the impeller mixing performance and act as cavities controlling suspension and dispersion mechanisms. Moreover, the periodic and fluctuating nature of trailing vortices accumulate most of the energy dissipated in the system leading to increased levels of turbulence and shear (Cutter, 1966).

Trailing vortex stability can be a function of impeller type, operating flow regime (Re), bioreactor working volume and geometry. Even though trailing vortices at higher Re are relatively stable and develop in the impeller swept volume as a consequence of blade passage, they can be a source of low frequency instabilities (macroinstabilities, MI) due to their interaction with other parts of the tank. Roussinova et al. (2003) investigated the MIs in a small and large scale baffled tanks equipped with a PBT via LES and found agreement on the characteristic frequency and distribution of the MI ($f_{MI} = 0.186N$) across the different scales (Roussinova, Kresta and Weetman, 2003). In another work, Roussinova et al. (2000) investigated MI in reactors equipped with various axial impellers and a RT and found that the periodicity of the generated flow field was associated with the number of baffles: fewer baffles led to disruption of the periodicity of the mean velocity time series. This was more pronounced in the radially embedded agitated tanks (Roussinova, Grgic and Kresta, 2000).

*Some of the results presented in this chapter are included in:


Charalambidou, A. D., Ducci, A., Micheletti, M. (2021) “Numerical and experimental investigation of the flow generated by a flat blade impeller used in perfusion processes”, 10th International Symposium in Mixing in Industrial Processes, Kobe, Japan
As detailed in chapter 1 several studies have examined in the past the trailing vortices emanating by RTs and FBTs using visualisation techniques based on the calculation of the maximum vorticity magnitude (Derksen and den Akker, 1999; Delafosse et al., 2009), the maximum absolute value of the negative eigenvalue of velocity gradient tensor, according to a method developed by (Jeong and Hussain, 1995) (Escudié, Bouyer and Liné, 2004; Başbuğ, Papadakis and Vassilicos, 2017) and the estimation of the second invariant of the velocity gradient tensor according to Q-criterion \(Q_{cr} = \frac{1}{2}(\Omega_{ij}\Omega_{ij} - S_{ij}S_{ij}) > 0\) (Zamiri and Chung, 2018; Arosemena, Ali and Solsvik, 2022).

In this chapter the trailing vortices developed inside the 250 mL custom made bioreactor, described in Section 2.2 and presented in Fig. 2.1 (b) and Fig. 2.1(c), are examined and the impact of baffle presence, size (B1<B2) and impeller disk on their stability is discussed. Trailing vortices were identified and visualised based on the magnitude of the tangential component of vorticity and velocity vector plots while their evolution was assessed according to instantaneous and phase resolved data from both simulations and experiments. Fluctuations of the trailing vortex core were quantified by measuring the oscillations of the angle of the impeller jet defined by the ratio between the axial and radial velocity components in the spatial window where the trailing vortices were located.

Frequency analysis of the jet fluctuation was performed by applying Proper Orthogonal Decomposition (POD) on the instantaneous velocity field of the 60-revolutions long LES dataset, the experimental validation of which is presented in chapter 3, and on the phase resolved PIV dataset (500 revolutions). Flow decomposition revealed the existence of highly energetic and periodic modes associated with interactions between impeller jet and reactor walls. These modes led to an impeller jet instability which is amplified by the baffle presence and size as well as the absence of impeller disk. In the present chapter characterisation of trailing vortices and impeller jet instabilities are investigated firstly for three configurations of the 250 mL custom made vessel equipped with a FBT (Fig. 2.1(a) and Fig. 2.1(b)): one unbaffled (UB) and two baffled configurations with baffles of different sizes (B1, B2). Results are presented in Sections 4.2.1-4.2.2. The impact of impeller disk on the impeller jet instabilities and trailing vortex formation is evaluated for a similar vessel configuration, shown in Fig. 2.1(c), equipped with a RT and three equally spaced baffles with a width of B1=\(D_T/16\). Results and discussion are presented in Section 4.2.3-4.2.4.
4.2 Results

4.2.1 Characterisation of trailing vortices with a FBT

Considering the impact of the trailing vortices on the STRs overall flow and mixing performance, a thorough characterisation of their formation and stability has been conducted in the following part of this work for the STR under investigation at small scale.

4.2.1.1 Vorticity magnitude

The phase resolved vector field and corresponding tangential vorticity, \( \langle \xi_\theta \rangle = D_T \langle \omega_\theta \rangle / \pi N \) contour map is shown in Fig. 4.1, based on LES data, for four different phase angles behind the blade (\( \varphi = 10^\circ, 20^\circ, 30^\circ \) and \( 40^\circ \)) and for all the three configurations UB, B1 and B2. In the unbaffled vessel the trailing vortices are clearly developed with nearly equal vorticity of opposite magnitude on either side of the impeller centreline. The two vortices are parallel and move radially outwards as the phase angle, \( \varphi \), increases. The addition of the smaller baffles B1 in the system introduces an inclination of the jet in between the vortex pair, which is increasing as the phase angle is increased. The jet inclination, \( \beta \), is quantified based on eq. 4.1 inside an area with dimensions \( 0.2<r/D_T<0.43 \) and \( 0.23<z/D_T<0.45 \) on the vertical plane of measurement (either \( P_1 \) or \( P_2 \)) for each configuration (i.e. UB, B1 and B2). For example, the centreline jet inclination, \( |\beta| \), computed from eq. 4.1 for B1, is found to reach approximately \( 4^\circ \) when \( \varphi = 10^\circ \) and \( \sim 5^\circ \) when \( \varphi = 40^\circ \), while it is \( \sim 0^\circ \) for the unbaffled case. This effect is even more pronounced for the larger baffle configuration, B2, where the jet inclination increases to \( |\beta| \sim 5^\circ-7^\circ \) for \( \varphi = 10^\circ-40^\circ \). Besides the inclination of the vortex pair and their central jet, the presence of baffles causes a more prominent radial movement of the bottom vortex as opposed to the top one. For example, in the B1 configuration the radial position of the bottom vortex ranges from \( r/D_T = 0.22 \rightarrow r/D_T = 0.32 \), as opposed to the UB vessel where the radial distance covered from the same vortex is \( r/D_T = 0.22 \rightarrow r/D_T = 0.27 \) when \( \varphi = 10^\circ-40^\circ \). These two effects, i.e. increased jet inclination and more prominent radial movement of the bottom trailing vortex, might be related to a reduction in trailing vortex stability as the blade progresses.

A 3D visualisation of the trailing vortices generated behind the blade for the FBT is shown in Fig. 4.2 for the three vessel configurations studied (UB, B1 and B2), where the trailing vortices are identified from isovorticity surfaces with a threshold of \( \langle \xi_\theta \rangle = D_T \langle \omega_\theta \rangle / u_{tip} = \pm 10 \) for the UB and the baffled configurations. 3D vorticity visualisation is illustrated both for an entire rotation and one blade passage. As suggested by the magnitude of tangential vorticity presented in Fig. 4.1 the two trailing vortices developed below and above the impeller
centreline are emanating from the blade in parallel for the UB vessel. With the addition of baffles the bottom vortex starts to expand outwards covering the top one. This determines the onset of the jet inclination between the two trailing vortices which appears to be enhanced with baffle size, as discussed in Fig. 4.1.

To get a better understanding of the way trailing vortices are evolving behind the blade, vorticity magnitude corresponding to different instants was calculated at 10° and 30° behind the blade (\(\phi=10^\circ, \phi=30^\circ\)). The resulting 2D instantaneous contour maps are vertically stacked in Fig. 4.3 for 500 revolutions (500 instants) taken from the PIV phase resolved dataset for the UB, B1 and B2 configurations. In the UB configuration the upper and lower trailing vortices are clearly defined and are parallel to each other, for both \(\phi=10^\circ\) and \(30^\circ\) across all the frames. When baffles are added, fluctuations of the centres of trailing vortices are observed. Their periodicity and intensity is influenced by the baffle size and phase angle, \(\phi\). An initial estimation of the frequency of the periodic fluctuations was implemented by measuring the distance between two consecutive peaks at \(\phi=30^\circ\) as indicated in Fig. 4.3(c). The peak to peak distance was estimated approximately \(\sim 8-10\) s and \(\sim 15\) s corresponding to an average of \(\sim 37\) \((f\sim 0.1\) Hz) and \(\sim 60\) \((f\sim 0.07\) Hz) impeller revolutions for configurations with B1 and B2 respectively.

The time variation of the minimum and maximum vorticity magnitude, \(\langle \xi_\theta \rangle\), in the axial direction \((z\text{-axis})\) was monitored and is illustrated in Fig. 4.4 based on phase resolved PIV data for all three reactor configurations studied and for three different phase angles \(\phi=10^\circ, 30^\circ\) and \(50^\circ\). Minimum and maximum vorticity values were estimated in a window \(0.2<r/D_T<0.33\) and \(0.23<z/D_T<0.45\) determined based on the phase averaged vorticity shown in Fig. 4.1 and this was kept consistent across the unbaffled and baffled configurations. The amplitude of the fluctuation of the trailing vortices was measured by estimating the standard deviation from the mean trajectory as indicated by the dashed lines in each plot in Fig. 4.4. In the UB case, in accordance with previous observations from Fig. 4.2 and Fig. 4.3, both vortices, upper and lower, are positioned in parallel while their interference is low and limited at \(\phi=50^\circ\). Similarly to Fig. 4.3 the addition of baffles introduces periodic fluctuations in the axial evolution of the trailing vortex core increasing their amplitude almost two-fold. For example, at \(\phi=10^\circ\), in the UB configuration the upper vortex fluctuates \(z/D_T \sim 0.39\pm 0.01\) while for the baffled vessels \(z/D_T \sim 0.39\pm 0.021\) and \(z/D_T \sim 0.39\pm 0.026\) for B1 and B2 respectively. This denotes a double increase in the amplitude of fluctuation with B1 and a further increase of \(\sim 20\%\) with B2. In addition the amplitude of fluctuation increases with blade angle across all configurations.
studied (unbaffled and baffled) indicating the loss of stability of the trailing vortices as the blade progresses. For instance \( z/D_T \approx 0.39\pm0.01 \) and \( z/D_T \approx 0.39\pm0.019 \) for the upper vortex in the UB tank at \( \varphi = 10^\circ \) and \( 50^\circ \) respectively (i.e. \( \sim90\% \) increase). Likewise, \( z/D_T \approx 0.39\pm0.021 \) and \( z/D_T \approx 0.39\pm0.039 \) for the upper vortex for the baffled configuration with B1 (i.e. \( \sim85\% \) increase) while a \( \sim55\% \) increase in amplitude was observed for the baffled vessel with B2. These data are summarised in Table 4.1.

The frequency of the fluctuation of the axial position of the trailing vortices was estimated for the baffled configurations by implementing an Fast Fourier Transform (FFT) analysis of the time vector of the axial positions across the different \( \varphi \). The estimated frequency ranged \( 0.09 \text{ Hz} < f < 0.11 \text{ Hz} \) and \( 0.06 \text{ Hz} < f < 0.07 \text{ Hz} \) for B1 and B2 respectively which is similar to the fluctuation frequency estimated in Fig. 4.3 from the peak-to-peak distance of instantaneous vorticity time series.

In Fig. 4.5 a comparison of vorticity levels is shown across the three vessel configurations studied in this work. To account for the expansion of trailing vortices, vorticity levels are compared based on the mean positive and negative values, processed from LES data, in proximity to the blade in a region defined between \( 0.2 < r/D_T < 0.43 \) and \( 0.23 < z/D_T < 0.45 \). For all vessel configurations the maximum absolute vorticity magnitude occurs when \( \varphi \approx 12^\circ \). In the UB tank the peak is at \( \varphi = 9^\circ \) while it shifts to angular positions further away from the blade, \( \varphi = 10^\circ \) and \( 12^\circ \), in baffled tank configurations, B1 and B2, respectively. Furthermore, the intensity of both vortices (upper and lower) increases in magnitude as configurations with baffles are considered with B2 being characterised by a nearly double absolute vorticity peak, when compared to the UB configuration (i.e. 8 (B2) versus 4 (UB) for top vortex) while a 30\% increase observed between B2 and B1 (i.e. 8 (B2) versus 6 (B1) for top vortex). Such behaviour is expected as both phase resolved vorticity magnitude and area covered by the trailing vortices appear to be larger and therefore more distributed in the B2 vessel. Finally, when comparing the upper and lower vortices for the B2 configuration, it is clear that there is an imbalance between the two, and the magnitude of the top vortex is nearly \( 17\% \) higher than the bottom one, as opposed to the UB and B1 configurations where the two trailing vortices exhibit similar magnitudes.

### 4.2.1.2 Analysis of FBT jet inclination

To further elucidate the trailing vortices dynamics, instantaneous velocity vector plots \( (u_i(\varphi, t)) \) denoted by the maximum and minimum inclination of the jet between the vortex pair are provided in Fig. 4.6 for all the configurations investigated. The vector maps, shown
in Fig. 4.6 include both LES (Fig. 4.6(a)) and PIV (Fig. 4.6(b)) datasets and are all related to a phase angle $\varphi=10^\circ$ at different time instants, when the trailing vortex cross-section is fully visible to the side of the blade. It is interesting to note that these large deflections of the impeller jet are only partially captured in the phase resolved data at $\varphi=10^\circ$, and are most likely to be more pronounced at larger phase angles where the imbalance between the two trailing vortices in the phase resolved vector maps is higher (as shown in Fig. 4.4 and Table 4.1 the amplitude of fluctuation measured via the standard deviation increased two-fold when the blade angle increases from $\varphi=10^\circ$ to $\varphi=50^\circ$).

To quantify the amount of jet fluctuation and identify potential frequencies related to this phenomenon, the angle of the jet in proximity to the impeller blade was estimated based on the ratio between the axial and radial velocity components (eq. 4.1), in a window with dimensions $0.2<r/D_T<0.43$ and $0.23<z/D_T<0.45$ on the vertical plane of measurement for each configuration studied. The size of the area was based on the location of trailing vortices from both instantaneous and phase averaged data (Fig. 4.1 and Fig. 4.3) and its use for the calculation of the angle of the jet was consistent across all vessel configurations for both simulations and experiments. For the UB vessel, the angle of the jet $|\beta|$ was estimated almost $\sim0^\circ$, confirming that the trailing vortices are parallel and located at similar positions across the different instants when $\varphi=10^\circ$. With the addition of baffles the jet inclination according to the instantaneous vector plots of Fig. 4.6, appears increased with slightly higher intensity manifested at B2 case. For example, $|\beta| \sim 26^\circ$-$28^\circ$ and $|\beta| \sim 24^\circ$-$33^\circ$ for B1 and B2 respectively, as indicated from the experimental dataset. Results showed consistency between simulations and experiments.

$$\beta(\varphi, t) = atan^{-1}\left(\frac{u_z}{u_r}\right)$$ \hspace{1cm} (4.1)

The results presented in Fig. 4.7 are based on instantaneous velocity phase resolved datasets obtained for $\varphi=10^\circ$, $30^\circ$ and $50^\circ$ from PIV, i.e. 500 frames corresponding to 500 revolutions of the impeller, for plane P1. To improve the readability of the data, the solid black reference line was obtained through a moving average, applied to smooth the dataset. For the unbaffled vessel, and for $\varphi=10^\circ$-$50^\circ$, it is evident that the fluctuations of the jet inclination are minimal, as the smoothed reference line marginally fluctuates around $0^\circ$. For the baffled tanks, the fluctuations are more pronounced with inclination of the reference black line varying between $\pm19^\circ$. Moreover, in the presence of baffles the jet inclination fluctuations are characterised by a periodic behaviour with a period ranging from 37 to 47 ($\sim 0.087$ Hz$<f<0.11$ Hz) revolutions.
and 57 to 68 (~0.06 Hz < f < 0.073 Hz) revolutions for the B1 and B2 vessel configurations, respectively. The amplitude of the fluctuation increases as larger phase angles are considered for both B1 and B2 cases. For example, when considering the B1 configuration the amplitude of the reference line is 9°, 12°, 16° for $\phi = 10^\circ$, 30° and 50° respectively, indicating that the instability is greater further away from the blade.

To study whether the oscillation amplitude and frequency was affected by the location of the baffles, the variation of the angle of the jet is shown in Fig. 4.8 for configuration B1 on the plane $P_2$, in proximity to the baffle, for both experiments and simulations. When comparing the phase resolved results from the PIV dataset in Fig. 4.7 and Fig. 4.8 it is evident that similar jet periodicity is occurring for the experimental data on both planes $P_1$ and $P_2$, with a frequency of ~37-47 revolutions which corresponded to $f \sim 0.087-0.11$ Hz, while the amplitude of oscillation is slightly higher in the $P_2$ plane, where $|\beta|$ ranges from 17°-19° at $\phi = 10^\circ$, 30° and 50°. To investigate whether the frequency of the jet fluctuation was consistent between the simulations and phase resolved PIV measurements, the time resolved 60-revolutions long LES dataset was further analysed for the B1 configuration. In this case, the angle of the jet was estimated per blade passage on the measurement plane $P_2$ (taking into account 360 positions in total for each angle $\phi$, i.e. every 60° for 60 revolutions) and is illustrated in Fig. 4.8. The estimated jet instability provided a peak to peak period of 8-10 impeller revolutions corresponding to a frequency of $f \sim 0.4-0.5$ Hz, four times higher than the one observed experimentally. The higher frequency values resulted from the LES can be related to the limited simulation time compared to the experiments (15 s versus 120 s) which is not long enough to fully resolve an instability characterised by a frequency as low as $f \sim 0.087-0.11$ Hz (observed experimentally). To elucidate these discrepancies, the flow fields for the UB and B1 configurations were further investigated via POD and FFT analyses for both experimental and computational datasets.

4.2.2 POD analysis and flow reconstruction for a FBT

To better understand the dynamics and nature of the jet instabilities and assess the periodicity of the oscillating frequency, POD was applied to the time resolved LES (simulation time corresponded to 20 impeller revolutions for the UB and baffled configurations with B2 and 60 impeller revolutions for the baffled configuration with B1) and PIV data (10 impeller revolutions), for all the vessel configurations. The percentage contribution of each mode to the total kinetic energy spectrum is illustrated in Fig. 4.9(a) and Fig. 4.9(b) where the modal energy is directly related to the corresponding eigenvalue. To consistently compare PIV and
LES data, velocity decomposition was conducted considering only velocity components in the axial and radial directions. The first mode contains most of the energy of the system (~6%) for all three configurations. This mode corresponds to the ensemble averaged flow field (i.e. the mean flow was not subtracted from the data set prior to applying POD). The most significant contribution to the total energy is observed from the first five modes as they appear to represent ~25% of the total energy. Modes of higher order (roughly after mode 10) tend to follow a -11/9 slope, denoted by the dash blue line in Fig. 4.9, which can be used as a reference to identify the range of modes corresponding to the turbulent inertial subrange (Liné et al., 2013). Comparison of the eigenvalue spectrum produced by LES and PIV in Fig. 4.9 shows good agreement between simulations and experiments and highlights that the current LES data is sufficiently resolved to correctly identify the turbulent inertial subrange. Previous CFD works based on RANS simulations which attempted to resolve the energy spectrum via POD, were denoted by a discrepancy with experimental results, with a steep decline of the energy content in the range of modes associated with the inertial subrange (de Lamotte et al., 2018b).

FFT was performed to the temporal coefficient $a_n(t)$ to find dominant frequencies of the POD modes. A visualisation of the energy content of the frequencies most commonly found in the three different tank configurations after conducting LES is illustrated in the bar plot in Fig. 4.10. The total energy of each frequency was computed by adding the energy associated with those modes, which exhibited the same dominant frequency after applying FFT on the temporal eigenfunction. As expected for each reactor type, the frequency with the highest energy corresponds to the blade passage frequency (BPF), $f_{BPF}=25$ Hz which is inherently linked to the periodic fluctuations and organised motion of the flow in proximity to the impeller. The next high energy content frequency, which is related to the second group of most energetic modes, corresponds to $f_{Baffled,LES}=0.416$ Hz and $f_{UB,LES}=2.29$ Hz for the baffled (with B1 and B2) and unbaffled configurations, respectively. Interestingly the second POD mode was always associated with those dominant frequencies identified for each configuration (i.e. 0.416 Hz for baffled and 2.29 Hz for UB).

To further elucidate the velocity fields and energy spatial distribution of the modes associated with those frequencies, the mode magnitude, $\sqrt{(\Phi_r(x))^2 + (\Phi_z(x))^2}$, is presented in Fig. 4.11. Fig. 4.11(a) illustrates the spatial eigenfunction of the first mode for both unbaffled and baffled reactors. As the first mode corresponds to the mean flow field, it captures most of the variance and is structurally similar to the ensemble averaged flow field (Liné et al., 2013). In Fig. 4.11(c) the eigenfunction corresponding to mode 3, with $f_{BPF}=25$ Hz, for both baffled and UB
configurations is representative of the organised flow motion produced by the impeller blade passage. In all configurations the flow associated with mode 3 is characterised by two high energy regions, on opposite sides of the impeller centreline for the UB case, which can be clearly related to the trailing vortices. For the UB and B1 configurations this is more apparent, while for the B2 case the lower trailing vortex region is shifted radially outwards, in agreement with the results presented in Fig. 4.1. Eigenfunctions of mode 2 ($f_{BAFFLED,LES}=0.416$ Hz and $f_{UB,LES}=2.29$ Hz) are illustrated in Fig. 4.11(b). The resulting structures indicate the presence of a stream from the tank wall towards the blade region where it sharply turns backwards towards the wall. This stream inverts its direction when there is a sign change (i.e. positive or negative) of the corresponding temporal coefficient $\alpha_n(t)$, resulting in a periodic motion from the wall to the impeller centreline. Modes 2 are structurally similar across all the reactor configurations investigated. In the unbaffled vessel the jet structure expands between $r/D_T\sim0.25$ to $r/D_T\sim0.4$ introducing a jet oscillation away from the impeller stream (Fig. 4.11(b)) while with the addition of baffles, this jet structure increases in intensity and moves closer to the impeller suggesting that the tank walls and baffle size play a major role on this organised motion.

To evaluate discrepancies related to the frequency analysis between LES and PIV and assess the nature of the instabilities arising from both datasets, POD was applied to the experimental phase resolved dataset for the UB and the B1 configurations for different phase angles ($\varphi=10^\circ$-$50^\circ$). The spatial eigenfunction associated with the second mode for the UB and B1 configurations is provided in the first and second column of Fig. 4.12 for $\varphi=20^\circ$ and $30^\circ$, respectively. From these two plots it is evident that a similar flow structure already identified by the LES POD analysis is also found from the PIV data, with a stream which emanates from the wall towards the impeller region and returns to the tank wall. For the UB configuration this structure is slightly smaller than the one found from the corresponding LES dataset (Fig. 4.11(b) and Fig. 4.12(e)). When the temporal eigenfunctions are considered, mode 2 of the unbaffled configuration exhibits a dominant frequency in the range of $1.7\,Hz<f_{UB,PIV}<2.1\,Hz$ for all the different phase angles examined $\varphi=10^\circ$-$50^\circ$. This is in agreement with the frequency, $2.29\,Hz$ found from LES, indicating that the simulations were long enough to capture the time scale of the instability oscillations ($4.8\,s$ corresponds to 9-10 cycles) for the UB configuration. For the B1 baffled vessel at the plane of measurement $P_2$, the decomposition resulted in a range of lower frequencies $0.087\,Hz<f_{B1,PIV}<0.11\,Hz$ for $\varphi=10^\circ$-$50^\circ$, which is consistent with the results of Fig. 4.7, Fig. 4.8(a), Fig. 4.8(c) and Fig. 4.8(e) and the analysis of Fig. 4.3 for the same
dataset. Therefore, for the B1 configuration the POD analysis applied to the LES results is capable of identifying the spatial flow structure, but due to the limited simulation time the associated frequency is overestimated.

To examine the impact of the jet instability expressed by the second POD mode on the average flow field, both experimental (Fig. 4.13 and Fig. 4.14) and computational (Fig. 4.15) datasets were reconstructed following eq. 2.45. The experimental phase resolved dataset for UB and B1 was reconstructed for all the different phase angles $\varphi = 10^\circ - 50^\circ$ considering the temporal modes corresponding to the frequencies associated with mode 2, lying in the range $1.7 \, \text{Hz} < f_{UB,PIV} < 2.1 \, \text{Hz}$ for UB and $0.087 \, \text{Hz} < f_{B1,PIV} < 0.11 \, \text{Hz}$ for the baffled vessel with B1. Indicative results for $\varphi = 20^\circ$ and $30^\circ$ are presented in Fig. 4.13 and Fig. 4.14 for the UB and the B1 configuration, respectively. For the simulation data, the flow field in all three configurations (UB, B1 and B2) was reconstructed where the temporal modes embed their corresponding frequencies of $f_{UB,LES} = 2.29 \, \text{Hz}$ and $f_{Baffled,LES} = 0.416 \, \text{Hz}$, respectively, and is presented in Fig. 4.15. To identify the effect of the flow structures associated with those frequencies on the average flow field, the first mode, representing the mean flow, was also added in the reconstruction process (for both simulation and experimental data). The results presented in Fig. 4.13-4.15 correspond to different instants of the reconstructed flow field and show that, even though the reconstructed velocity field structurally resembled the average flow field due to the higher energy of the first mode, as the impeller stream separates the velocity field into two circulation loops, an oscillation of the impeller jet is induced in all configurations. In both experimental and computational data, for the UB reactor, the reconstructed velocity vector plots showed that the impeller stream is quite steady and a downward inclination is induced with $f_{UB,LES} = 2.29 \, \text{Hz}$ and $f_{UB,PIV} = 1.9$ and $2.1 \, \text{Hz}$ (for $\varphi = 30^\circ$ and $20^\circ$, respectively), at $r/D_T > 0.27$ (Fig. 4.13, Fig. 4.14 and Fig. 4.15). With the addition of baffles the frequency of the jet instability is reduced to $f_{Baffled,LES} = 0.416 \, \text{Hz}$ (for baffled vessels with B1 and B2) and $f_{B1,PIV} = 0.11$ and $0.087 \, \text{Hz}$ (for $\varphi = 20^\circ$ and $30^\circ$, respectively) causing a more intense jet fluctuation initiated closer to the impeller blade ($r/D_T \approx 0.2$), where the trailing vortices emanate. The jet inclination of the reconstructed flow field was estimated following eq. 4.1 and was consistent with the one defined from the phase resolved dataset illustrated in Fig. 4.7 and Fig. 4.8 corresponding to $|\beta| \approx 15^\circ - 17^\circ$.

The results shown in this work are consistent with those of Roussinova et al. (2000) who studied the effect of baffles on flow stability and reported a large impact on flow periodicity in systems agitated with RTs when number of baffles was reduced from four to two.
According to their work, four-baffle configuration led to higher flow stability because of the formation of strong circulation loops which were generated by the increased drag imposed by the baffles (Roussinova, Grgic and Kresta, 2000). Additionally in another work Roussinova et al. (2003) studied the macroinstabilities inside a baffled tank equipped with a four-blade PBT and reported three factors that can trigger them. The first two are associated with the impingement of the impeller jet on the tank walls and the flow deflection by the baffles whereas the third is linked to the trailing vortices (Roussinova, Kresta and Weetman, 2003). In their study the impeller clearance was \((C/D_t \sim 0.6)\), which is close to the one used in this work \((C/D_t \sim 0.5)\). They reported that the flow deflection was a consequence of a pressure impingement at the corner of the tank due to an imbalance formed between the flow diverted towards the tank wall and that towards the bottom of the tank. The mechanism described by Roussinova et al. (2003) can be linked to the jet instability found in this work and can ultimately amplify the effect of flow impingement on the reactor wall creating an oscillating jet which becomes more intense with baffle presence and their increase in size. The reduced stability of trailing vortices may also be attributed to the absence of an impeller disk. In the presence of a FBT and with the current operating conditions \((Re=3,732)\) and working volume \((250 \text{ mL})\) trailing vortices seem to roll up near the blades, while their radial propagation and expansion is lower compared to studies investigating a RT (Stoots and Calabrese, 1995; Escudié, Bouyer and Liné, 2004). Hence, the weak vortex propagation, the operating conditions and the impeller clearance, are all parameters that can potentially affect trailing vortex stability, which may be more pronounced when SDMs are used.

### 4.2.3 Trailing vortices and jet inclination with a RT

The impact of impeller disk on the stability and propagation of the trailing vortices was examined in the vessel configuration illustrated in Fig. 2.1(c) (Chapter 2) with working volume of 250 mL and three equally spaced baffles with a width of \(B_1 = D_T/16\). Phase resolved data (for \(\varphi=10^\circ, 20^\circ, 30^\circ\) and \(40^\circ\)) for the tangential vorticity magnitude \(\langle \xi_\theta \rangle = D_T \langle \omega_\theta \rangle / u_{\text{tip}}\) and the corresponding velocity vector plots based on a 40-revolution long LES are presented in Fig. 4.16. Similarly to the data illustrated in Fig. 4.1 for the UB vessel with a FBT, the trailing vortices are clearly developed on either side of the impeller centreline and move radially outwards as the blade angle increases. Contrary to previous observations for the baffled configuration with \(B_1\) (Fig. 4.1) at \(\varphi=30^\circ\) and \(40^\circ\), the jet inclination is not evident in the presence of a RT (Fig. 4.16) and the two trailing vortices remain parallel to each other for all the phase angles presented. Moreover, when comparing the RT with the FBT for the baffled
vessel with B1 the upper vortex moves further out in the presence of RT. This is a consequence of the absence of the jet inclination which prevents the acceleration of the bottom vortex and its interference with the top one. For example, in the presence of the FBT, the position of the upper vortex ranges from \( r/D_T = 0.22 \) to \( r/D_T = 0.25 \) whereas it is \( r/D_T = 0.22 \) to \( r/D_T = 0.3 \) (\( \phi = 0^\circ - 40^\circ \)) for the same vessel configuration, i.e. baffled with B1, when it is equipped with a RT.

A 3D visualisation of the trailing vortices generated behind the blade for a RT is shown in Fig. 4.17 for the baffled bioreactor with B1 (Fig. 2.1(c)). The isovorticity surfaces in this case were used with a threshold of \( \langle \xi_\theta \rangle = \pm 15 \) and are presented for an entire impeller revolution and a blade passage. As illustrated in Fig. 4.16, the two counter-rotating trailing vortices are separated by the impeller disk and move parallel to each other (Bouremel, Yianneskis and Ducci, 2009b). In addition their surface is curved backward and propagates radially outward when moving away from the impeller blade in a more pronounced way compared to the FBT (Fig. 4.2).

Staggered plots of the instantaneous vorticity magnitude for two phase angles (\( \phi = 10^\circ \) and \( 30^\circ \)) are presented in Fig. 4.18 for the vertical plane P1. Results are extracted from LES data (simulation time of 40 revolutions) and show instantaneous tangential vorticity magnitude corresponding to the same \( \phi \) (i.e. taking into account 240 positions in total for each angle \( \phi \)). For both phase angles presented (\( \phi = 10^\circ \) and \( 30^\circ \)) the trailing vortices are clearly defined and move parallel to each other, while fluctuations of the trailing vortex centres (mainly evident at \( \phi = 30^\circ \)) are much lower in intensity compared to the FBT (Fig. 4.3). An initial estimation of the period of those fluctuations was estimated by measuring the peak to peak distance between two consecutive maximum and minimum values, as indicated in Fig. 4.18, and corresponded to \( \sim 1.8-1.9s \) (\( f \approx 0.5-0.6 \text{ Hz} \)). In Fig. 4.19 2D contour maps of the phase resolved vorticity magnitude (\( \langle \xi_\theta \rangle \)) are vertically stacked for 24 phase angles between two consecutive blade passages (i.e. \( \phi = 0^\circ - 60^\circ \) with a step of 2.5°) for the baffled vessels with B1 equipped with both radial impellers used, i.e. RT and FBT. In the case of the FBT (Fig. 4.19(b)) the intensity of trailing vortices is weaker than in the RT, while for blade angles \( \phi > 30^\circ \) the vorticity magnitude drops significantly indicating that their propagation is limited compared to the RT. Specifically, for the FBT and B1 configuration (Fig. 4.1) maximum vorticity magnitude for the bottom vortex at \( \phi = 30^\circ \) and \( 40^\circ \) is approximately two times lower than that of the RT (e.g. \( \langle \xi_\theta \rangle \sim 15 \) and \( 32 \) at \( \phi = 30^\circ \) for the FBT and the RT respectively).

A comparison of vorticity levels across all four vessel configurations studied in the current chapter, is shown in Fig. 4.20. Similarly to Fig. 4.5, in Fig. 4.20 the intensity of vorticity
magnitude based on the mean positive and negative values in proximity to the impeller blade is presented according to LES data within $0.2 < r/D_T < 0.45$ and $0.2 < z/D_T < 0.48$. For the bottom vortex, the absolute maximum vorticity occurs at similar phase angles when compared to the baffled (B1) case equipped with a FBT ($\varphi = 11^\circ$ in the vessel with B1 and RT versus $\varphi = 10^\circ$ in the vessel with B1 and FBT) but the intensity of vorticity magnitude is almost double in the presence of a RT. For example, for the baffled vessel with B1 $\langle \xi_\theta \rangle \sim 11$ and 6 in the presence of a RT and FBT respectively. For the top vortex at the same vessel configuration (baffled with B1) the maximum vorticity magnitude is slightly shifted when the RT is used ($\varphi \sim 11^\circ$ versus $\varphi \sim 7^\circ$ for RT versus FBT) but the absolute maximum value is similar for both impellers ($7 < \langle \xi_\theta \rangle < 8$). Nevertheless, when comparing the RT and the FBT for the baffled vessel with B1, the absolute maximum for both trailing vortices is consistently higher for all blade angles when the former is used.

Several studies in the past have focused on the analysis of the trailing vortices emanating from a RT in baffled vessels (Nienow and Wisdom, 1974; Riet and Smith, 1975; Yianneskis, Popiolek and Whitelaw, 1987; Schäfer, Höfken and Durst, 1997; Escudié, Bouyer and Liné, 2004; Delafosse et al., 2009). As previously mentioned, 3D visualisation of trailing vortices presented in Fig. 4.17 for the RT and in Fig. 4.2 for the FBT, was implemented by using a threshold of $\langle \xi_\theta \rangle \sim \pm 15$ (higher than $\langle \xi_\theta \rangle \sim \pm 10$ used for the FBT case), which is close to $\langle \xi_\theta \rangle \sim \pm 13$ used by Schäffer et al. (2000) but lower than the values reported by Delafosse et al. (2008) ($30 < \langle \xi_\theta \rangle < 95$) for a similar impeller type in a fully baffled vessel of larger volume ($V \sim 70 \text{ L}$, at $Re \sim 55,000$) (Schäfer, Höfken and Durst, 1997; Delafosse et al., 2009). In another work Bouremel et al. (2009) discussed the evolution of the trailing vortices emanating from a RT and reported a threshold $\langle \xi_\theta \rangle \sim 9$ for a $\sim 2 \text{ L}$ fully baffled vessel operating at $Re \sim 2,800$ (Bouremel, Yianneskis and Ducci, 2009b). Discrepancies in vorticity magnitude and trailing vortex characteristics between the current work and literature may be attributed to differences in reactor working volume and operating flow regime. Nevertheless, the evolution of the trailing vortices emanating from a RT are in agreement with the cited literature (Schäfer et al., 2000; Yeoh, Papadakis and Yianneskis, 2004; Bouremel, Yianneskis and Ducci, 2009b) while the threshold difference between RT and FBT corresponding to $\langle \xi_\theta \rangle \sim 15$ and $\langle \xi_\theta \rangle \sim 10$ respectively, highlights the intensification of vorticity when RT is used. The high vorticity magnitude and its fixed location across the different instants for the same phase angle lead to the development of vortical structures with increased stability compared to those emanating from the FBT.
To further understand the dynamics of the trailing vortices emanating from a RT and compare their stability to those of a FBT, instantaneous velocity vector plots at different $\varphi$ ($u_i(\varphi, t)$) were examined and the jet inclination between the vortex pair was estimated according to eq. 4.1 within the window $0.2<r/D_T<0.45$ and $0.2<z/D_T<0.48$, determined based on the phase resolved vorticity data (Fig. 4.16). Indicative results are presented in Fig. 4.21 where velocity vector plots are illustrated at different instants for $\varphi=10^\circ$ and $30^\circ$ based on LES. Similarly to the discussion of the Section 4.2.1.2 for the FBT, the jet fluctuations increase with blade position. For example, indicative values for jet inclination are in the range of $4^\circ<|\beta|<15^\circ$ and $6^\circ<|\beta|<23^\circ$ when $\varphi=10^\circ$ and $\varphi=30^\circ$ respectively. However, it is interesting to note that most of the jet deflections occur at radial distances $r/D_T>0.3$ which is further away from the area where the trailing vortices are formed (trailing vortex formation window is $\sim0.2<r/D_T<0.3$) for these phase angles.

### 4.2.4 POD analysis and flow reconstruction for a RT

To better understand the dynamics of the instabilities associated with the jet fluctuations generated from the RT, POD was applied to the time resolved LES data (40 revolutions) extracted for the corresponding vessel configuration (Fig. 2.1(c)). Similarly to Section 4.2.2 decomposition of the velocity flow field was implemented based only on the velocity components corresponding to the axial and radial direction, while FFT was performed to the temporal coefficient $a_n(t)$ to find dominant frequencies of the POD modes. The percentage contribution of each mode to the total kinetic energy spectrum is illustrated in Fig. 4.22 combined with the vessel configurations equipped with a FBT. It is evident that the energy spectrum for both impellers is captured from the POD eigenvalues in a similar way, with the first mode of the RT curve containing 8% of the total energy and higher modes (> 10) following the -$11/9$ slope corresponding to turbulence.

The spatial distribution of the first three modes, containing the highest amount of energy, is presented in Fig. 4.23 based on the mode magnitude, $\sqrt{(\Phi_r(x))^2 + (\Phi_z(x))^2}$, for the vertical plane in proximity to the baffle $P_2$. Results calculated for the RT are compared to those corresponding to FBT, both extracted from LES datasets. Similarly to the case of the FBT, for the RT the mean was not subtracted from the dataset prior to POD therefore the first mode (Fig. 4.23(a)) corresponds to the average flow field and has a frequency $f=0$ Hz while it contains most of the system energy ($\sim8\%$ of the total energy versus $6\%$ of the total energy represented from the FBT). For the RT, mode 2 (Fig. 4.23(b)) is characterised by $f=25$ Hz which
is representative of the BPF ($f_{BPF}=25$ Hz) and is associated with two high energy regions on either side of the impeller centreline corresponding to the trailing vortices. With the RT the trailing vortices seem to be fully separated and developed on opposite sides of the impeller disk. On the contrary, when the FBT was used Fig. 4.23(c), the trailing vortices appear as merged structures developing around the impeller centreline. For the RT the eigenfunction of mode 3 is illustrated in Fig. 4.23(c) and is associated with a frequency $f_{B1,LES,RT}=0.52$ Hz. The resulting structure indicates the presence of a stream similar to that one illustrated for mode 2 of the FBT ($f_{B1,LES,FBT}=0.416$ Hz) shown in Fig. 4.23(b) but of lower intensity. Moreover, this jet structure expands within $0.25<r/D_T<0.45$ as opposed to $0.2<r/D_T<0.4$, evident in the case of FBT, indicating that the corresponding jet instability occurs at a larger distance compared to the FBT case and is less energetic. The lower amount of energy carried from this instability in the RT case can be manifested by its lower ranking in the energy cascade (appearing at mode 2 versus mode 3 for the FBT and RT respectively) and quantified by estimating its total energy considering the modes exhibiting similar dominant frequencies ($f_{B1,LES,RT}=0.52$ Hz) based on FFT results on the temporal eigenfunction (i.e. energy of the instability was estimated for the RT $\lambda_i/\sum \lambda_i \sim 4\%$ and $\sim 5\%$ for the FBT case as indicated in the bar plot of Fig. 4.10).

The impact of the jet instability, in the presence of a RT, expressed by the third POD mode on the average flow field was examined for the plane in proximity to the baffle ($P_2$) by reconstructing the corresponding LES dataset according to eq. 2.45. Therefore, the modes associated with $f_{B1,LES,RT}=0.52$ Hz were added to the average flow field, represented by mode 1 ($f=0$ Hz) and the results are presented in Fig. 4.24 where each plot corresponds to different instants of the reconstructed flow field ($t_1<t_2<t_3$). For comparison purposes the first row corresponds to the reconstructed flow fields for the FBT ($f$ (mode 1, $f_{mean}=0$ Hz) + $f$ (mode 2, $f_{B1,LES,FBT}$ (0.416 Hz)) whereas the second row corresponds to the RT ($f$ (mode 1, $f_{mean}=0$ Hz) + $f$ (mode 3, $f_{B1,LES,RT}$ (0.52 Hz)). In both cases the reconstructed flow field is similar but the existing oscillations in the RT case appear less intense with the jet inclination of the reconstructed flow field corresponding to $|\beta|\sim 5^\circ-8^\circ$ (according to eq. 4.1) as opposed to $|\beta|\sim 15^\circ-17^\circ$ found for the FBT. Moreover, the jet oscillations appear to be initiated at $r/D_T>0.25$ as opposed to $r/D_T \leq 0.2$ in the FBT, which limits the interference of the instability with the trailing vortices. The frequency of mode 3 ($f_{B1,LES,RT}=0.52$ Hz) in the presence of RT is in agreement with the frequency range discussed in Fig. 4.18 ($f\sim 0.5-0.6$ Hz) estimated based on the peak to peak distance of the amplitude of oscillation of the trailing vortex core evident.
at $\varphi=30^\circ$. It is worth noting that the frequency of mode 3 associated with the jet oscillation ($f_{B1,LES,RT}=0.52$ Hz) in the RT case corresponds to only five cycles of the jet instability as the LES lasted for 40 impeller rotations. As discussed before in Section 4.2.2 LES dataset might not be long enough to fully resolve the frequency range of this instability but gives meaningful insight on the spatial distribution of the resulting instability.

The spatial distributions of the modes corresponding to the three principal frequencies ($f_{mean}=0$ Hz, $f_{BPFF}=25$ Hz and $f_{B1,LES,RT}=0.52$ Hz) after applying POD for the baffled tank (B1) with a RT, are illustrated in Fig. 4.25 for the horizontal plane at $z/D_T\sim0.39$ corresponding to the centreline of the RT, based on the LES dataset. As discussed in Fig. 4.23 and Fig. 4.11 the first POD mode (Fig. 4.25(a)) represents the mean flow field ($f_{mean}=0$ Hz) and is localised at the impeller rotating zone with the velocity vectors indicating impeller rotation in the clockwise direction. Modes 2-4 corresponded to the blade passage frequency ($f_{BPFF}=25$ Hz) and are linked to the organised motion produced by the blade passage, which is representative of the trailing vortices. Indicative results for the spatial distribution of those are illustrated in Fig. 4.25(b) for mode 2, where 12 high energy regions are evident corresponding to the trailing vortices (two for each impeller blade). Modes 5-7 had a frequency of $f_{B1,LES,RT}=0.52$ Hz and are illustrated in Fig. 4.25(c), Fig. 4.25(d) and Fig. 4.25(e). For these modes the region of high energy is shifted to the stationary zone of the tank away from the impeller rotation and is localised closer to the vessel wall. It is interesting to note that each one of these modes exhibits a high energy area in proximity to each baffle. For example the spatial distribution of mode 5 shown in Fig. 4.25(d), reveals a high energy region ($|\Phi|\sim0.03$) close to the bottom left baffle whereas, for mode 6 and 7 (Fig. 4.25(d) and (e)) magnitude is higher in proximity to the top and bottom right baffles, respectively.

To study the impact of modes 5-7 on the average flow field and elucidate their association with the baffle presence, the velocity field was reconstructed for the horizontal plane at $z/D_T\sim0.39$ considering the first mode (corresponding to the mean) and modes 5-7. The results are presented in Fig. 4.26 for five different instants $t_1<t_2<t_3<t_4<t_5$. As expected the reconstructed velocity is higher at the centre of the plane as most of the energy of the system is generated from the impeller rotation and is localised at the centre of the tank. Nevertheless, the flow reconstruction revealed the existence of three elements (A, B and C shown in Fig. 4.26) which are formed in proximity to each of the baffles and move clockwise in the different instants. The frequency of modes 5-7 is equal with the frequency of mode 3 ($f_{B1,LES,RT}=0.52$ Hz) found on the vertical plane in proximity to the baffle B1 (P2), which is responsible for the
fluctuation of the impeller jet as indicated in Fig. 4.24. This indicates that modes 5-7 for the horizontal plane and mode 3 for the vertical plane are associated with the presence of baffles and that the latter can be assigned frequencies that can affect the average flow field depending on their energy content. As previously mentioned, the determination of a specific frequency associated with the jet instability presented in this work can only be approximated by LES as more impeller revolutions should be simulated in order to accurately resolve the temporal characteristics of such instability. However, LES can give a good approximation for the spatial distribution and the amount of energy of the POD modes associated with the presence of baffles.

The above analysis conducted for both impeller types (i.e. FBT and RT) shows that flow decomposition in baffled stirred tanks reveals modes related to the baffle presence which can be a source of low frequency instabilities. The energy of those modes depends on the bioreactor and impeller configuration and can determine the intensity of the resulting instabilities. In the case of the FBT, the instability associated with the trailing vortex fluctuation appeared in mode 2, at the vertical plane in proximity to the baffle, and its energy was high enough ($\lambda_i/\sum \lambda_i \approx 5\%$ of the total variance) to periodically change the location of the trailing vortex centre (Fig. 4.11-4.15). In the presence of impeller disk (i.e. RT) the resulting instability appeared in modes 3 and 5-7 for the vertical and horizontal planes respectively (Fig. 4.23(c) and Fig. 4.25(c), Fig. 4.25(d) and Fig. 4.25(e)) and the reduced intensity had lower impact on the fluctuation of the trailing vortex centre (Fig. 4.24, $|\beta| \approx 5^\circ-8^\circ$ with RT versus $|\beta| \approx 15^\circ-17^\circ$ with FBT). The presence of impeller disk seemed to stabilise the formation of trailing vortices, while combined POD results indicated that the impeller stream evident in mode 3 (Fig. 4.23(c)) and associated with the jet fluctuation, was linked to flow perturbations localised in proximity to the baffles.

POD and flow reconstruction has been discussed in the past for stirred and shaken vessels (Doulgerakis, Yianneskis and Ducci, 2011; Liné, 2016; de Lamotte et al., 2018b; Weheliye et al., 2018; Mayorga, Morchain and Liné, 2022). Most of the previous works applied POD aiming at the investigation of the higher order modes associated with the impeller motion in order to extract the main flow structures and reduce the dimensionality of the data (Ducci, Doulgerakis and Yianneskis, 2008; Rodriguez et al., 2013; de Lamotte et al., 2018b). Some other works focused on the investigation of turbulent components and turbulent length scales via POD (Liné, 2016). Investigation of modes of higher order and discussion of their association with the baffles after flow reconstruction is limited. Mayorga et al. (2021) applied POD on the 3D
flow field generated by CFD RANS simulations for a fully baffled tank (4 baffles) equipped with a RT. In their work, flow decomposition revealed the existence of clockwise vortices behind each baffle, appearing after mode 3, with frequency four times higher than the impeller frequency (Mayorga, Morchain and Liné, 2022). In the same context, flow investigation and decomposition was conducted in a shaken square reactor (Li, 2019). POD results showed that spatial distribution of modes 1 and 2 corresponded to highly energetic structures in the centre of the shaken reactor whereas modes 3 and 4 revealed four high energy regions corresponding to each corner of the squared vessel (unpublished data, (Li, 2019)). These results imply that the corners of a square (or prismatic) tank may have a similar effect to impact on the flow to baffles (Chung, Simmons and Barigou, 2009; Fan et al., 2021).

The development and evolution of trailing vortices in radially agitated tanks has been discussed in the past for both RT (Nienow and Wisdom, 1974; Riet and Smith, 1975; Yianneskis, Popiolek and Whitelaw, 1987) and FBT (Winardi and Nagase, 1994). Winardi et al. (1993) studied the trailing vortices produced by a FBT and suggested that the flow properties near the vortex region are similar to those coming from a Rushton Turbine in baffled tanks but the separation mechanism of the trailing vortices is different between the two impellers. In baffled vessels when RTs are used, the formation of trailing vortices occurs at the leading (i.e. front) edge of the blade. In the case of FBT, the separation point is moved towards the interior part of the blade (i.e. closer to the impeller shaft), due to the absence of impeller disk and blade extension up to the impeller shaft. Such alterations in the separation mechanisms, change the position of the low pressure region associated with the trailing vortex centre and influence other parameters such as the power number ($P_0$) which is ~10-20% reduced in the case of FBTs compared to RTs (Bates, Fondy and Corpstein, 1963). In the current work, $P_0$ in both vessel configurations (Fig. 2.1(b) and (c)) was estimated from LES based on the average moment acting on the impeller shaft according to eq. 2.2 and eq. 2.3 and corresponded to 4.8 and 5.6 for the vessel equipped with FBT and RT respectively (i.e. ~16% decrease in $P_0$ between the two impellers) which is in agreement with (Bates, Fondy and Corpstein, 1963).

Yianneskis et al. (1987) experimentally investigated the trailing vortices emanating from a RT in a baffled tank and correlated their propagation to several tank design parameters including impeller diameter and clearance. They reported that increasing the $C/D_T$ led to a decrease in the maximum velocities in the impeller stream, but increased the spread of the trailing vortices. In addition, they discussed the impact of decreasing $C/D_T$ on the impeller stream inclination and suggested that the latter increased from 2.5° to 7.5° when $C/D_T$ decreased.

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from 0.5 to 0.25, and attributed that behaviour to generation of pressure gradients (Yianneskis, Popiolek and Whitelaw, 1987). This is in agreement with Roussinova et al. (2003) who discussed the generation of instabilities due to flow impingement at the tank corners in a reactor stirred with a PBT and similar clearance (Roussinova, Kresta and Weetman, 2003). In the current study, the impeller clearance is similar to the aforementioned works \((C/DT \sim 0.3)\) and may ultimately lead to amplification of flow impingement on the vessel walls creating an oscillating jet which becomes more intense with baffle presence and their increase in size.

The impact of impeller disk on the propagation and stability of trailing vortices has been studied in the current work by comparing the levels of vorticity magnitude and the intensity of the jet instabilities between a FBT and a RT for the same configuration. The results presented in this chapter and the comparison between the two radial impellers (FBT versus RT) underlines the impact of impeller characteristics on the generated flow field and highlights the flow sensitivity in the operating conditions and tank geometry which may be more pronounced in small scale reactors.

4.3 Summary

In this chapter thorough investigation of trailing vortices was conducted across different vessel configurations (i.e. unbaffled versus baffled) and different radial impeller types (FBT versus RT). Analysis of the impact of baffle presence and size on the evolution and stability of trailing vortices is presented in Sections 4.2.1 and 4.2.2. The impact of impeller disk is discussed in Sections 4.2.3 and 4.2.4 where trailing vortices were characterised in the presence of a standard RT in the 250 mL custom made vessel (Fig. 2.1) equipped with three equally spaced baffles with size B1.

In the case of the FBT, characterisation of trailing vortices was implemented in terms of the magnitude of tangential vorticity estimated from both LES and PIV data across three vessel configurations including unbaffled and baffled vessels with different baffle sizes \((B1<B2)\). Phase resolved data showed that in the UB case the impeller trailing vortices were clearly defined, had equal magnitude and moved radially outwards and in parallel as the blade angle increased. In the presence of baffles the parallel movement of the vortices became weaker due to an impeller jet inclination which was more intense with increasing baffle size. The impeller jet inclination was estimated based on the ratio of axial and radial velocity components for both simulation and experiments and appeared to be stable in the UB tank, leading to the formation of two distinctive trailing vortices, while in the baffled vessels periodic oscillations
were induced which amplified with increasing baffle size. The periodicity of the jet fluctuation corresponded to 37-47 revolutions \((f \sim 0.087-0.11 \text{ Hz})\) and 57-68 revolutions \((f \sim 0.06-0.073 \text{ Hz})\) for the B1 and B2 configurations, respectively. These frequencies could be resolved with PIV experimental data which were long enough to fully capture the instability time scale.

To elucidate instabilities affecting trailing vortex formation, spatio-temporal velocity data were processed with POD to find principal modes of variation along with FFT to assign the most prominent frequencies to each mode. Flow decomposition revealed two frequency ranges associated with the flow stream interacting between the tank wall and impeller flow region. These frequencies corresponded to the second most energetic modes and were related to similar flow disturbance effects on the mean velocity flow field. The impact of these periodic flow structures on the mean flow was studied by a Low Order Model (LOM) comprised only by the most energetic modes. These reconstructed models clearly indicated the presence of a jet instability which was amplified in baffled systems, and was associated with a sharp flow stream emanating from the wall and interfering with the impeller flow region.

Trailing vortex intensity and stability were increased when the FBT was replaced with a RT in the baffled vessel with B1. Tangential vorticity magnitude was consistently higher (two-fold increase) across all the blade angles \((\varphi=0^\circ-50^\circ)\) investigated and indicated that the trailing vortices expanded in parallel from \(\varphi=0^\circ\) to 50\(^\circ\). Flow decomposition in the presence of the RT showed that the mode associated with the jet instability was of weaker intensity due to its lower order in the energy cascade (mode 3 in RT versus mode 2 in the FBT) and induced a jet inclination of low amplitude \(|\beta| \sim 5^\circ-8^\circ\) in the RT versus \(|\beta| \sim 15^\circ-17^\circ\) in the FBT) and at higher radial distances minimally interfering with the impeller trailing vortex region. Flow reconstruction with the first mode (representative of the mean) and those modes related to this instability led to fluctuations with reduced intensity compared to the FBT and revealed the existence of three elements in proximity to the baffles, which moved clockwise across the different instants.

The analysis presented in the current chapter underlines the impact of reactor geometry and impeller type on the development of trailing vortices and generation of flow instabilities. Flow sensitivity on the baffle size and impeller type, indicates the importance of parameter optimisation when small scale STRs are tested and highlights the significance of detailed understanding of the hydrodynamics for optimising design features.
Figure 4.1: Contour map of normalised vorticity ($\langle \xi_\theta \rangle = D_T(\omega_\theta)/u_{tip}$) for UB and baffled configurations (B1 and B2 at plane $P_1$) for phase angles ranging $\varphi = 10^\circ$-$40^\circ$. Results presented correspond to LES dataset. Plots for vorticity magnitude are presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 4.2: 3D visualisation of the trailing vortices for the UB, B1 and B2 equipped with FBT and associated a threshold value of: $\langle \xi_\theta \rangle = -10$ (blue) and $\langle \xi_\theta \rangle = 10$ (red) configurations for the vertical plane plane $P_1$. Results correspond to LES data.
Figure 4.3: Staggered contour maps of the instantaneous vorticity, $\xi_\theta(\varphi, t) = D_T\omega_\theta(\varphi, t)/u_{\text{tip}}$, for (a) $\varphi=10^\circ$ and (b) $\varphi=30^\circ$ for the UB and baffled configurations (B1, B2) at plane $P_1$. In (c) a close up of the stagger plots at $\varphi=30^\circ$ are indicated for all configurations studied (UB and baffled with B1 and B2). Results correspond to phase resolved PIV data.
Figure 4.4: Evolution of the axial position of the trailing vortices based on minimum and maximum vorticity magnitude ($\xi = D_T \omega_\theta(\phi, t)/u_{tip}$) calculated from the phase resolved PIV dataset for (a) $\phi = 10^\circ$, (b) $\phi = 30^\circ$ and (c) $\phi = 50^\circ$. Continuous lines indicate the average axis of the trajectory of the top and bottom trailing vortices (illustrated in blue and red respectively) and the dashed lines indicate the standard deviation from their mean trajectory. Data correspond to vertical planes of measurement for UB and baffled configurations (B1 and B2). For the baffled configurations the vertical plane is $P_1$. 
Table 4.1: Quantification of the fluctuation of the upper vortex based on the standard deviation from the mean vortex trajectory. Data correspond to Fig. 4.4.

<table>
<thead>
<tr>
<th>phase angle ($\varphi$)/vessel configuration</th>
<th>$\varphi=10^\circ$</th>
<th>$\varphi=30^\circ$</th>
<th>$\varphi=50^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unbaffled (UB)</td>
<td>0.39 +/-0.01</td>
<td>0.39 +/-0.015</td>
<td>0.39 +/-0.019</td>
</tr>
<tr>
<td>Baffled, B1=$D_T/16$</td>
<td>0.39 +/-0.021</td>
<td>0.37 +/-0.034</td>
<td>0.37 +/-0.039</td>
</tr>
<tr>
<td>Baffled, B2=$D_T/10$</td>
<td>0.39 +/-0.026</td>
<td>0.37 +/-0.039</td>
<td>0.38 +/-0.04</td>
</tr>
</tbody>
</table>
Figure 4.5: Vorticity level based on local spatial averages of LES data at plane $P_1$ for the unbaffled (continuous line) and baffled configurations, B1 (Dashed line) and B2 (Dotted line): (a) upper vortex and (b) lower vortex. Plots are presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 4.6: Instantaneous \((u_i(\varphi, t)/u_{tip})\) and phase resolved \((\langle u_i \rangle / u_{tip})\) velocity vector plots for \(\varphi=10^\circ\) and for plane \(P_1\): (a) LES and (b) PIV. Red arrows indicate the jet inclination. Plots are presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 4.7: Variation of the angle of the jet, \( \beta \), at plane \( P_1 \) for the B1 and B2 configurations. The jet angle was estimated from phase resolved PIV data (every revolution) for phase angle: (a) \( \varphi=10^\circ \), (b) \( \varphi=30^\circ \) and (c) \( \varphi=50^\circ \). Plots are presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 4.8: Variation of the angle of the jet, $\beta$, at plane $P_2$ for the B1 configuration. The jet angle was estimated from phase resolved PIV (every revolution) and LES (every impeller passage) data for phase angles $\varphi=10^\circ$ ((a) and (b)), $30^\circ$ ((c) and (d)) and $50^\circ$ ((e) and (f)). Plots are presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 4.9: POD eigenvalue spectrum: (a) from LES for the three vessel configurations (UB and baffled with B1 and B2 at plane P2) and (b) for B1 baffled tank from PIV and LES data (P₂ plane). Plots are presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 4.10: Cumulative eigenvalue contribution associated with the peak frequencies found after performing FFT analysis of POD modes from LES dataset ($P_2$ plane of measurement). Plot is presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 4.11: Magnitude of the first three POD modes for the three vessel configurations at the plane of measurement $P_2$ (LES dataset): (a) mode 1 ($f = 0$ Hz), (b) mode 2 ($f_{UB,LES} = 2.29$ Hz, $f_{B1,LES} = 0.416$ Hz and $f_{B2,LES} = 0.416$ Hz for the UB and baffled configuration with B1 and B2 respectively), (c) mode 3 ($f_{BPF} = 25$ Hz). Plots are presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 4.12: Magnitude of the second POD mode obtained from PIV and LES data for the UB and baffled configuration with B1. Contour plots correspond to the magnitude of the second POD mode after POD was applied to both PIV phase resolved data ((a), (b), (c) and (d)) and LES instantaneous data ((e) and (f)). (e) and (f) contours are taken from Fig. 4.11(b). Plots for $\varphi=20^\circ$ are presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 4.13: Magnitude of the second POD mode (first column) and contours of the reconstructed velocity field (columns 2 and 3) based on PIV phase resolved dataset for (a) $\varphi=20^\circ$ and (b) $\varphi=30^\circ$ for the UB configuration. For both phase angles flow reconstruction was performed considering (a) $f_{UB, PIV} (mode\ 1, f_{mean, PIV} = 0\ Hz) + f_{UB, PIV} (2.1\ Hz)$ and (b) $f_{mode\ 1, f_{UB, PIV, mean} = 0\ Hz} + f_{UB, PIV} (1.9\ Hz)$. The different contour maps for the reconstructed velocity field correspond to two different instants, $t_1 < t_2$. White dotted lines and arrows indicate the point where jet inclination begins and its direction respectively. Plots for $\varphi=20^\circ$ are presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 4.14: Magnitude of the second POD mode (first column) and contours of the reconstructed velocity field (columns 2 and 3) based on PIV phase resolved dataset for (a) $\varphi=20^\circ$ and (b) $\varphi=30^\circ$ for the baffled configuration with B1. For both phase angles flow reconstruction was performed considering (a) $f_{B1, PIV, mean}=0$ Hz and $f_{B1, PIV}=0.11$ Hz and (b) $f_{B1, PIV, mean}=0$ Hz and $f_{B1, PIV}=0.087$ Hz. The different contour maps for the reconstructed velocity field correspond to two different instants, $t_1 < t_2$. White dotted lines and arrows indicate the point where jet inclination begins and its direction respectively. Plots for $\varphi=20^\circ$ are presented in Charalambidou, Micheletti and Ducci, (2022).
Figure 4.15: Contour and vector maps the instantaneous flow field ($t_1 < t_2 < t_3$) reconstructed with modes containing the two most dominant frequencies. For the UB STR reconstruction was implemented with $f$ (mode 1, $f_{\text{mean}} = 0$ Hz) + $f_{\text{UB,LES}}$ (2.29 Hz). Results are presented for vertical plane $P_2$ (B1 and B2) and correspond to LES dataset. For the baffled tanks reconstruction was implemented with $f$ (mode 1, $f_{\text{mean}} = 0$ Hz) + $f_{\text{Baffled,LES}}$ (0.416 Hz). White dotted lines and arrows indicate the point where jet inclination begins and its direction respectively. Plots are presented in (Charalambidou, Micheletti and Ducci, 2022).
Figure 4.16: Contour map of normalised vorticity \((\langle \xi_\theta \rangle = D_T (\omega_\theta) / u_{tip})\) ranging \(\varphi=10^\circ-40^\circ\) for the bioreactor configuration equipped with B1 and a RT. Results presented correspond to LES dataset at the vertical plane \(P_1\).
Figure 4.17: 3D visualisation of the trailing vortices for the bioreactor configuration equipped with B1 and a RT. Blue and red vortices correspond to $\langle \xi_\theta \rangle = -15$ (blue) and $\langle \xi_\theta \rangle = 15$ (red). LES data are presented at the vertical plane $P_1$. 
Figure 4.18: Staggered contour maps of the instantaneous tangential vorticity magnitude, 
\( \xi_\theta(\phi, t) = D_T \omega_\theta(\phi, t) / u_{tip} \), for (a) \( \phi = 10^\circ \) and (b) \( \phi = 30^\circ \) for the baffled bioreactor configuration B1 and a RT. Results correspond to LES dataset and are presented for the P_1 plane of measurement.
Figure 4.19: Staggered contour maps of the phase resolved vorticity, $\langle \xi_\theta \rangle = D_1(\omega_\theta)/u_{tip}$, for increasing $\varphi = 0-60^\circ$ for the baffled bioreactor configuration with B1 and (a) a RT and (b) a FBT. Results correspond to LES dataset and are presented for the $P_1$ plane of measurement.
Figure 4.20: Vorticity level based on local spatial averages of LES data at plane $P_1$ for the bioreactors equipped with both FBT and RT. For the FBT, curves are illustrated as follows: unbaffled (continuous line) and baffled configurations, B1 (Dashed line) and B2 (Dotted line). For the RT, baffled vessel with B1 is presented (symbol x). For all reactor configurations: (a) upper vortex and (b) lower vortex.
Figure 4.21: Instantaneous ($u_i(\phi, t)/u_{tip}$) and phase resolved ($u_i/u_{tip}$) velocity vector plots for (a) $\phi=10^\circ$ and (b) $\phi=30^\circ$ for the bioreactor configuration equipped with B1 and a RT. Red arrows indicate the jet inclination. LES data are presented for the vertical plane of measurement $P_1$. 
Figure 4.22: POD eigenvalue spectrum corresponding to LES dataset for four vessel configurations: UB and baffled with B1 and B2 and the configuration with B1+RT. Data are presented at the vertical plane $P_2$. 
Figure 4.23: Magnitude of the first three POD modes for the B1 configurations equipped with FBI and RT (LES dataset). For the FBT (a) mode 1 ($f = 0$ Hz), (b) mode 2 ($f_{B1,LES,FBT} = 0.416$ Hz), (c) mode 3 ($f_{BPFF} = 25$ Hz). For the RT mode 1 ($f = 0$ Hz), (b) mode 2 ($f_{BPFF} = 25$ Hz), (c) mode 3 ($f_{B1,LES,RT} = 0.52$ Hz).
Figure 4.24: Contour and vector maps the instantaneous flow field \((t_1 < t_2 < t_3)\) reconstructed with the modes associated with the jet instability for B1 configuration with FBI and RT. For the STR with B1 and a FBT reconstruction was implemented with \(f \text{ (mode 1, } f_{\text{mean}} = 0 \text{ Hz)} + f_{B1,LES,FBT} \) (0.416 Hz). For the STR with B1 and a RT reconstruction was implemented with \(f \text{ (mode 1, } f_{\text{mean}} = 0 \text{ Hz)} + f_{B1,LES,RT} \) (0.52 Hz).
Figure 4.25: Magnitude of the first three POD modes for the B1 configurations equipped with a RT (LES dataset). Results are presented for the horizontal plane at the impeller centreline ($z/D_T = 0.39$): (a) mode 1 ($f = 0$ Hz), (b) modes 2-4 ($f_{BPF} = 25$ Hz), (c)-(e) modes 5-7 ($f_{B1,LES,RT} = 0.52$ Hz).
Figure 4.26: Contour and vector maps of the instantaneous flow field ($t_1 < t_2 < t_3 < t_4 < t_5$) reconstructed with modes containing the two most dominant frequencies for the bioreactor configuration equipped with a RT (LES dataset). Results are presented for the horizontal plane at the impeller centreline ($z/D_T = 0.39$). Reconstruction was implemented with $f$ (mode 1, $f_{mean} = 0$ Hz) + $f_{B1,LES,RT}$ (0.52 Hz).
Chapter 5: Investigation of the impact of bioreactor internals on power and flow

5.1 Introduction

The biopharmaceutical industry faces continuous pressure to reduce process development costs and decrease time to market. This has enhanced efforts towards intensification of upstream experimentation and investigation of robust and predictive models for systems biology, following quality by design principles (Bareither and Pollard, 2011). In this context small scale (mL-scale) systems, such as miniature bioreactors, have been widely adopted within the pharmaceutical industry both as screening tools and Scale-Down Models (SDMs) operating under GMP in manufacturing processes (Sandner et al., 2019).

For SDMs to be reliable and efficient, equivalence of the critical quality attributes (CQAs) and performance parameters should be demonstrated and preserved across the different scales. Nevertheless, scaling among bioreactors with different volumes and geometries can be challenging due to the resulting differences in mixing and flow that may significantly impact reactor performance and process efficiency if not thoroughly examined (i.e. lead to poor mixing and/or high shear environments) (Li et al., 2006).

*Some results presented in this chapter are included in:


Common well-established scaling criteria are associated with maintaining mixing time \( t_M \), oxygen transfer coefficient \( k_L a \) and power per unit volume \( P/V \) but these are qualified under the assumption of geometric similarity and operation in fully turbulent conditions.

Considering that most of the scaling parameters have been developed for pilot or larger scale reactors, it is important for these to be tested and expanded to smaller volume reactors, where the dimensions of probes and baffles relative to the reactor size can greatly impact the reactor flow field and scaling efficiency. In addition, as mentioned in previous chapters, small scale vessels are often designed to study and optimise process performance, resulting in unconventional configurations and sizing criteria compared to larger scale tanks (e.g. parallelepiped vessel configuration of ambr®15), which could significantly impact the overall process outcomes. Previous studies have shown that the small volume ranges in scale-down devices, may lead to a decrease in \( Re \) resulting in disparity in the operating conditions across scales and imposing additional challenges in scaling methodologies. Moreover, as discussed in chapter 1, it has been suggested that the presence of probes may act in an equivalent way to baffles when small scale vessels are examined (Nienow, Rielly, et al., 2013b; Collignon et al., 2016; Rotondi et al., 2021).

Thorough investigation of the impact of bioreactor internals number and geometry, including baffles and probes, on hydrodynamics and power characteristics has rarely been reported even though the latter serves as one of the principal criteria for scaling in the biopharmaceutical industry. Power input per unit volume \( P/V \) is a function of fluid properties, working volume, impeller size and rotational speed and is usually expressed via a non-dimensional parameter \( P_0 \) (Xing et al., 2009). In most of the industrial applications scaling procedures based on \( P/V \) rely on \( P_0 \) information available in the literature and/or values provided by the vendor. However, these values may not always be suitable for mL-scale bioreactors as the former are based on standardised vessels (baffled or unbaflled, 5 – 100 L or more), whereas for the latter \( P_0 \) values correspond to either the UB or the baffled configurations, while the presence of additional internals such as addition lines and probes are not considered.
In this chapter, the power number \((P_0)\) measured across different small scale bioreactors for axial and radial flow impellers and the impact of bioreactor internals, including baffles and probes, on \(P_0\) is investigated. Measurements were conducted in a 250 mL custom made bioreactor equipped with a FBT (Fig. 2.1) for both unbaffled (UB) and baffled configurations and the impact of two different probe sizes on power consumption was assessed (Fig. 2.1(d)). In addition, axial impellers were investigated experimentally in two commercial SDMs with different volumes, including the 60 mL EasyMax™ (Mettler-Toledo) equipped with a 45° PBT and the 1 L Univessel® (Sartorius) equipped with a 45° 3BS impeller. The impact of internals on \(P_0\) in both types of flow (axial and radial) was examined. Details on reactor geometry and internal sizing are described in chapter 2 (Section 2.2) and presented in Fig. 2.1-2.3 and Tables 2.1-2.3.

Reactor hydrodynamics were studied via CFD LES for a specific configuration only, namely the 250 mL custom made vessel equipped with a FBT, three equally spaced baffles \(B1 = D_T/16\) and two probes \(P_a\) of equal volume \((V_{Pa}=3.5 \text{ mL})\), shown in Fig. 2.1(d). The impact of probes on global velocity and turbulence was characterised. Moreover, vorticity and pressure distributions around the probe surface were investigated and used for the estimation of form and friction drag in order to understand the contribution of each to the systems power requirements. The flow patterns around the probe and pressure investigation were used to explore novel probe geometries, which promise to reduce power requirements.

5.2 Results

5.2.1 Power number curves for axial and radial impellers in small scale reactors

The power characteristics of the FBT in the 250 mL custom made bioreactor is presented in Fig. 5.1(a). Power number \((P_0)\) was measured experimentally in seven different configurations of this vessel including: an UB/No Internal (UB/NI) configuration, two baffled configurations with B1 and B2 (B1<B2), two configurations with probes of different sizes \((P_a, P_b\) with \(P_a<P_b\)) and no baffles and two configurations
with both probes and baffles B1 (P_a+B1 and P_b+B1). For all configurations, at \( Re<100 \) data are inversely proportional to \( Re \) and overlap irrespectively of the presence of baffles and/or internals. This is expected as at low \( Re \) energy is dissipated in proximity to the impeller and therefore is not affected by the presence of internals away from the impeller (Bates, Fondy and Corpstein, 1963). Consequently, the presence of baffles has minimal impact on \( P_0 \) in the laminar flow regime. At \( Re>100 \) the curves start diverging and at \( Re>4,000 \) a plateau is reached for the configurations with internals. It is worth noting that this value is close to the \( Re \) value at which flow experiments have been conducted (\( Re=3,732 \)) and presented in chapters 3 and 4. The fact that a plateau is reached at \( Re<10,000 \) indicates that for small scale reactors turbulent flow is developed at a lower Reynolds threshold when compared to the larger reactors (Nienow, Rielly, et al., 2013b).

In UB vessels due to intense circumferential motion present at high impeller speeds, the free surface of the liquid deforms and a central vortex is formed with a depth proportional to the agitation speed. When the depth of the vortex reaches the impeller plane (super-critical regime) bubbles are generated and dispersed in the liquid phase, which may potentially lead to cell damage due to bubble bursting. In the current work, in the UB bioreactors the impeller velocities used did not result in the super-critical regime (i.e. the central vortex did not reach the impeller plane) and data presented in Fig. 5.1 corresponds to a system operating in the sub-critical flow regime defined by Scargiali et al. (2017). According to their work \( P_0 \) can be expressed as a function of \( Re \) in the turbulent region (eq. 5.1) (Scargiali et al., 2017):

\[
P_0 = K \cdot Re^{0.3}
\]

(5.1)

where \( K \) depends on the vessel geometry and sizing. In the current work for the 250 mL custom made bioreactor agitated with a FBT (Fig. 5.1(a)) and at the turbulent regime (\( Re>5,000 \)) \( P_0 \) data followed the same trend with \( K \sim 21 \) (i.e. the above eq. is \( P_0=21 \cdot Re^{0.3} \)) as opposed to 19.5 (\( P_0=19.5 \cdot Re^{0.3} \)) reported by Scargiali et al. (2017) for an unbaffled vessel mixed with a RT with similar clearance (\( C/Dr \sim 0.3 \)) (Scargiali et al., 2017).
For the FBT, (Fig. 5.1(a)), at high $Re$, $P_0$ gradually increases when transitioning from a configuration with no internals to a configuration with either baffles or probes. As expected, $P_0$ is the lowest in the UB case (and decreases continuously with increasing $Re$) but with the addition of small probes, $P_a$ (with volume~3.5 mL each), the power number increases and stabilises in a similar way to a baffled tank. Moreover, higher $P_0$ values are observed when the larger probes, $P_b$, are used (with volume~7.9 mL each) while power number is further increased, though at a lower rate, in the presence of baffles with increasing size (B1, B2). The highest power number values ($P_{0,max}$) are reached in the presence of baffles B1 and probes $P_b$ ($P_b$+B1, $P_{0,max}$~6.4 for $Re$>5,000).

Impeller power characteristics have been computed via CFD LES at $Re$=3,732, for four reactor configurations i.e. the UB, baffled with B1 and B2 and the vessel with small baffles and probes ($P_a$+B1). The corresponding results at this $Re$ are summarised in Table 5.1. Experimental and computational data are in very good agreement for the reactor configurations with internals (i.e. baffled and baffled with probes) with the maximum error being ~5% for the baffled configuration with B2. For the UB configuration the error between experiments and simulations is higher (~30%), but such difference can be attributed to the fact that the deformation of the free surface (which is more evident in the UB case) is not accounted for in the simulations, as discussed in chapter 3. Nevertheless, simulations were always conducted in closed vessels (i.e. with a lid, surface is not free to move and deform) and therefore the resulting $P_0$ values are expected to be slightly higher than the experimental ones regardless of the presence of internals (Nienow and Miles, 1971; Scargiali et al., 2017). Taking into account the slight overestimation of $P_0$ values from LES due to the absence of the simulation of the free surface, the relative error values shown in Table 5.1 were calculated according to $100 \times \frac{|P_{0,experiment} - P_{0,CFD}|}{P_{0,experiment}}$ considering that in the experiments there were no such limitations and Power number values are representative of the realistic ones.

Power number values obtained experimentally at high $Re$ ($Re$>5,000), where $P_0$ versus $Re$ curve has reached a plateau, were compared with published data corresponding to the turbulent flow regime and the results are summarised in Table 5.2. Data are within
the range of those reported in the literature for baffled vessels with FBT with similar non-dimensional sizing parameters. For example, Bates et al. (1963) investigated the power number across different radial impellers including RTs and FBTs with various sizes. In their work they reported that for FBTs with $0.125 < w_b / D_i < 0.2$ the $P_0$ ranged within $3 < P_0 < 4$ but increased linearly with increasing $w_b / D_i$ with values being predicted within the range $5 < P_0 < 8$ when $0.25 < w_b / D_i < 0.4$ (Bates, Fondy and Corpstein, 1963). In the current work $w_b / D_i = 0.26$ and at high $Re$ ($Re > 5,000$) $P_0 \approx 5.1$ and 5.3 for baffled vessels with B1 and B2, respectively, which are within the range reported in the literature. Nevertheless, when comparing $P_0$ potential discrepancies between the literature and the reported data can be attributed to changes in baffle configurations (4 baffles of $B \approx D_T / 12$ in Bates et al. (1963) versus 3 baffles of $B1 \approx D_T / 16$ in the current work), sizing parameters and reactor geometry (i.e. clearance, blade thickness) that can greatly affect the resulting $P_0$ value (Major-Godlewksa and Karcz, 2018b).

Power number results for the 250 mL vessel were compared to commercial bioreactors with different working volumes (60 mL – 1 L) equipped with various types of axial impellers including a 45° PBT and a 45° 3BS. The experiments for those vessels have been conducted by two other members of the research group and are only used in this thesis for comparison purposes. The results are illustrated in Fig. 5.1(b) (experiments conducted by Dr Anne De Lamotte) and 5.1(c) (experiments conducted by Tom Wyrobnik and presented in Wyrobnik et al., (2021)). For the 60 mL EasyMax™ (Mettler-Toledo) $P_0$ gradually increases with the addition of internals and ranges between $1.2 < P_0 < 1.6$, which is in agreement with data presented in the literature (Nienow and Miles, 1971; Chapple et al., 2002; Major-Godlewska and Karcz, 2018b). In the case of the 1 L Univessel® (Sartorius), shown in Fig. 5.1(c), the bioreactor configuration with internals corresponds to a vessel equipped with six probes of various sizes, used for mammalian cell culture. It is interesting to note that when all internals are present the turbulent power number, $P_0$, increases ~2.8 times from the corresponding UB/NI case ($P_0 \approx 0.8$ versus $P_0 \approx 2.3$ for the UB/NI configuration and the configuration when all internals are present respectively). This is close to the increase observed for the 60 mL EasyMax™ vessel between UB/NI and fully baffled
configurations, indicating that when all internals are present, $P_0$ curve resembles a baffled tank ($P_0 \sim 0.5$ versus $P_0 \sim 1.4$ for the UB/NI and baffled configuration respectively).

It has been suggested that the presence of probes has an equivalent impact on bioreactor hydrodynamics and mixing to the presence of baffles (Rotondi et al., 2021). When looking at the $P_0$ versus $Re$ curve in Fig. 5.1, it is evident that at high $Re$ ($Re > 5,000$), $P_0$ becomes independent of $Re$, when probes are added in the vessels irrespective of the presence of baffles. This phenomenon seems to be more pronounced for the radial impellers as it is shown in Fig. 5.1(a) (i.e. at high $Re$ after the first probe addition, $P_0$ stabilises in the case of FBT, whereas it continues to decrease slightly in the case of axial impellers in Fig. 5.1(b) and Fig. 5.1(c)). The impact of internal type, i.e. probes and baffles, on power number was characterised according to results based on two of the SDMs with smaller volumes, presented in Fig. 5.1: the 250 mL custom made bioreactor (equipped with a FBT) and the 60 mL EasyMax™ (Mettler-Toledo, equipped with a PBT). The results are presented in Fig. 5.2 in which a close-up of $P_0$ versus $Re$ is presented at $Re > 5,000$ where changes in $P_0$ values due to internals are more evident. The influence of both internal types is studied separately for each configuration and in the case of probes, data are presented in terms of probe blockage ratio, defined as the ratio of the probe volume over the total fluid working volume ($V(\%)$). For the radial impeller and specifically for the custom made 250 mL bioreactor (Fig. 5.2(a)), the presence of small probes ($P_a$) with $V(\%) \sim 2.8\%$ leads to a $P_0$ increase of approximately 2.5 times, when compared to the UB case ($P_0 \sim 1.5$ in the UB versus $\sim 3.8$ in the presence of $P_a$). Furthermore, $P_0$ increases from 3.8 to 4.5 (corresponding to $\sim 20\%$ increase) when transitioning from $P_a(V(\%) \sim 2.8)$ to $P_b(V(\%) \sim 6.3)$, while the addition of the larger probes increases $P_0$ three-fold compared to the UB/NI scenario. Similarly, for the axially agitated vessel (Fig. 5.2(c)) the increase in $P_0$ is a function of the probe and/or internal size, but the power number appears to be less impacted by the first probe addition compared to the radially agitated vessel. For example, the addition of a single temperature probe (T probe) with $V(\%) \sim 2.3$ leads to a 1.6 times increase of $P_0$ from the corresponding UB/NI case ($P_0 \sim 0.5$ in the UB/NI case versus $P_0 \sim 0.8$ when T probe is added). A further 40%
increase is observed when both temperature and pH probes are present which corresponds to a $V(\%) \sim 8\%$. Finally, when all internals are present, i.e. probes and two addition lines for feeding and sampling ($V(\%) \sim 8.4\%$), $P_0$ is found to increase a further 10%, while it is $\sim 2.6$ higher than the corresponding UB/NI case. The aforementioned data for $Re > 5,000$ for both STRs are summarised in Table 5.2.

For the same vessels the impact of baffles on $P_0$ was isolated and correlated to the presence of probes and impeller type. The results are presented in Fig. 5.2(b) and Fig. 5.2(d) for the FBT and PBT, respectively. It is evident that in both bioreactors the addition of baffles results in higher $P_0$ compared to probes. For the 250 mL custom made vessel with a FBT (Fig. 5.2(b)) and for $Re > 5,000$, $P_0$ in the B1 vessel configuration is $\sim 35\%$ and $\sim 13\%$ higher than the $P_0$ for the same vessel when it is equipped only with $P_a$ or $P_b$, respectively. In the presence of both probes and baffles (i.e. $P_a+B1$ and $P_b+B1$) $P_0$ increases approximately 20% between B1 and $P_a+B1$ ($P_0 \sim 5.1$ in B1 versus $P_0 \sim 6$ in $P_a+B1$) and 25% between B1 and $P_b+B1$ ($P_0 \sim 5.1$ in B1 versus $P_0 \sim 6.4$ in $P_b+B1$).

In the case of the 60 mL EasyMax™ (Mettler-Toledo) with a PBT (Fig. 5.2(d)) $P_0$ is less affected by baffle addition as the transition between the vessel with all internals ($V(\%) \sim 8.4$) to the fully baffled configuration (4 baffles with $B=D_T/10$) results only in a 6% increase of $P_0$ for the latter (Table 5.2).

The above analysis indicates that the influence of internals, including both probes and baffles, on the $P_0$ is more significant for the radial impeller than for the axial one. In the case of probes, when all internals are present, lower $V(\%)$ leads to higher percentage increase of $P_0$ for the FBT compared to the PBT (i.e. $0<V(\%)<6.3$ increases $P_0$ three times for the FBT case compared to a 2.6 times increase in $P_0$ achieved when $0<V(\%)<8.4$ in the PBT case). Similarly, the impact of baffles on $P_0$ is greater for the FBT as the transition between UB/NI to a fully baffled vessel, such as the configuration with B2, leads to 3.5 and 2.8 times higher $P_0$ for the FBT and PBT, respectively. Moreover, switching from a configuration with probes to a configuration with baffles appears to marginally affect the PBT ($\sim 6\%$ increase in $P_0$ for the baffled vessel) but leads to a $\sim 20-30\%$ increase in $P_0$ for the FBT. It is interesting to note that based on comparison of the data between the Tables 5.1 and 5.2 for the FBT, in the
presence of probes the $P_0$ appears to be more impacted by changes in $Re$ than it seems to be in the standardised baffled configuration. For example, for the configurations with probes, including $P_a+B1$ and $P_b+B1$, $P_0$ continues to increase $\sim 20-25\%$ in the range $3,732<Re<5,000$ before plateauing at $Re>5,000$. On the other hand, for the baffled tanks (either with $B1$ or $B2$) the $P_0$ plateau seems to be reached at slightly lower $Re$ (even at $Re=3,732$) given that the estimated values remain nearly unaffected at $3,732<Re<5,000$. Those results demonstrate the sensitivity of $P_0$ to the vessel geometry, scale and flow regime and highlight the need to thoroughly assess it during scaling. The aforementioned analysis on the comparison among the different vessel configurations is summarised in Table 5.2 for both impeller types (FBT and PBT) for $Re>5,000$.

The greater sensitivity of the radial impellers on the presence of baffles has been reported by Godlewska et al. (2018) who investigated the impact of several arrangements of tubular baffles on $P_0$ for both axial and radial flows in STRs (Major-Godlewska and Karcz, 2018a). In their study, it was discussed that $P_0$ decreased with increasing clearance between the baffles and the tank wall while it was stressed that any modifications in tubular baffle geometry impacted mostly the power number of vessels stirred with RTs due to the direct interference of the internals with the resistance of the strong radial flow produced (Major-Godlewska and Karcz, 2018a). It is worth noting that based on Fig. 5.1 and Fig. 5.2 and data in Table 5.2, the measured $P_0$ in small scale STRs when all internals are present (i.e. probes and baffles) appears increased and deviates significantly from well-established empirical data depending on impeller type and flow pattern. For example for the FBT, when both probes and baffles were present, $P_0$ was estimated to be $\sim 30\%$ higher than values reported in the literature, which refer to standard baffled tanks equipped with similar impellers (i.e. $P_0\sim 6.4$ in $P_b+B1$ case versus $P_0\sim 5$ in standard vessels with $V=10-50$ L according to Bates et al. (1963)) (Bates, Fondy and Corpstein, 1963). For the 60 mL EasyMax$^\text{TM}$ (Mettler-Toledo) with the $45^\circ$ PBT, comparison of the measured $P_0$ with literature data showed lower increase of power number confirming the lower sensitivity of axial impellers on internal addition discussed previously and observed in Fig. 5.1(b) and Fig. 5.2 (i.e. $P_0$ of the vessel with probes or baffles was approximately $10\%$ and $16\%$, respectively,
higher than published data from Chapple et al., (2002) corresponding to a standard 10-L baffled tank).

When scaling occurs based on constant $P/V \propto P_0 N^3 D_i^2$ observed differences in $P_0$ will inevitably impact power per unit volume ($P/V$), as an underestimated power number obtained from a standard configuration (i.e. not accounting internals) will lead to an underestimation of shear stress and energy dissipation in small scale reactors resulting in dissimilarities in the flow between the large and small scale vessels. Nevertheless, the rate in which $P_0$ is increasing across the various internal additions (i.e. it reduces with increasing $V(\%)$ of probes and addition lines) can be correlated to the baffling effect described by Bates et al. (1963) and Lu et al. (1997) according to which a continuous increase in the number and/or size of baffles decreases their impact on $P_0$ but also might minimise their beneficial influence on mixing. For example, Bates et al. (1963) reported that depending on the impeller diameter, when $n_b w_b / D_T > 0.4$, where $n_b$ and $w_b$ are the baffle number and impeller blade width respectively, power number decreased 20-30% (Bates, Fondy and Corpstein, 1963). Moreover, Lu et al. (1997) after studying power characteristics in a radially agitated vessel at pilot scale, reported an increase in mixing time when excessive baffling was used: when $n_b > 8$ and $0.15 < B/D_T < 0.3$ mixing time increased by ~50% (Lu, Wu and Ju, 1997). The aforementioned sizing parameters for baffles, are comparable to the size and number of probes found in smaller scale vessels (according to Table 2.2, where ratios of probe to vessel diameter are presented) and are expected to become more significant with further reduction in bioreactor volume highlighting that the engineering characterisation of small scale systems is important to thoroughly understand the way design parameters affect the main scaling criteria.

5.2.2 Impact of probes on flow dynamics

The impact of probes on flow dynamics was investigated at $Re=3,732$ via CFD LES for the 250 mL custom made vessel equipped with a FBT, three equally spaced baffles with B1 size ($B1=D_T/16$) and two cylindrical probes of equal size ($P_{ar} V_{Pa} \sim 3.5$ mL)(presented in Fig. 2.1(d)).
5.2.2.1 Velocity and turbulence distribution

Mean and turbulent velocity distribution in the presence of probes $P_a$ (configuration with $P_a$+B1) have been studied with LES and compared with the corresponding unbaffled and baffled (with B1 and B2) reactor configurations presented in chapters 3 and 4. Contour maps of ensemble average velocity magnitude, $|u|/u_{tip}$ with superimposed vector fields are presented in Fig. 5.3 for both vertical planes, $P_1$ (in proximity to the probe) and $P_2$ (in proximity to the baffle) for the UB, B1, $P_a$+B1, and B2 configurations. As discussed in chapter 3, the in-plane velocity magnitude is the lowest in the UB case but appears to increase with the addition of baffles and/or probes and is the highest in the presence of the largest baffles B2. For example, the transition from the UB to the baffled configuration with B1 leads to an increase in velocity magnitude of $\sim 20\%$, while the addition of probes $P_a$ is found to further increase the maximum velocity by $\sim 15\%$ compared to the corresponding no-probes case. Similar percentage increase, (i.e. $\sim 15\%$) is observed between the $P_a$+B1 and the B2 vessels as $(|u|/u_{tip})_{\text{max}}$ is estimated to be $\sim 0.6$ and $0.7$, respectively. In addition it is evident from Fig. 5.3 that the width of the impeller discharge stream appears to be greater in the presence of internals (baffles and/or probes). This effect is more pronounced in $P_a$+B1 and B2 configurations in proximity to the probe $P_a$ (plane $P_1$) and baffle B2 (plane $P_2$), respectively.

A direct comparison among the ensemble average turbulent characteristics of the different bioreactor configurations is presented in Fig. 5.4, where in-plane turbulent kinetic energy, $k'$ calculated by averaging the corresponding phase resolved values with $0.5^\circ$ resolution from LES dataset, is illustrated for both vertical planes $P_1$ and $P_2$. A gradual increase in $k'$ is evident with increasing number and size of internals i.e. $k'_{UB}<k'_{B1}<k'_{P_a+B1}<k'_{B2}$. The impact of baffle size on turbulent kinetic energy is clear as $k'$ appears to be the highest for the baffled vessel with B2. The transition between the vessels with B1 and $P_a$+B1, leads to a $\sim 20\%$ rise in the average $k'/u_{tip}^2$, which is attributed to an increase in the overall flow resistance and drag caused by the presence of probes, while $k'$ becomes $\sim 20\%$ greater between $P_a$+B1 and B2. Moreover, similarly to the observation on the distribution of $|u|/u_{tip}$ discussed in Fig. 5.3, the width of the region characterised by high $k'$ (at $0.22<r/D_T<0.3$), increases with internal number and
size and it is the highest in proximity to the baffle B2 (at plane P2). It is interesting to note that for the current regime (Re=3,732), regardless of the presence of probes, for both mean velocity and turbulent kinetic energy distributions the flow seems to be impacted more by the presence and size of baffles. This is in accordance with data presented in Table 5.1 indicating that power number values among the different vessels are ranked in a similar way, i.e. $P_{0,UB} < P_{0,B1} < P_{0,Pa+B1} < P_{0,B2}$, with the highest $P_0$ occurring for the baffled configuration with B2. Average kinetic energy ($k$) was compared with power number data by Wyrobnik et al. (2021) between a novel impeller configuration (Bach impeller) and a 45° 3BS axial impeller. In their work although average kinetic energy was of similar magnitude between the two impeller configurations ($k_{\text{max}} \sim 0.2u_{\text{tip}}^2$), maximum values expanded over a larger fluid volume surrounding the impeller area leading to 50% higher dissipation rate and consequently $P_0$ (Wyrobni et al., 2021).

In the literature the available information on the impact of reactor internals on the generated flow field is limited as the majority of studies have focused on the characterisation of standard vessel geometries either UB (Alcamo et al., 2005) or baffled configurations (Stoots and Calabrese, 1995; Lee and Yianneskis, 1998; Escudié and Liné, 2003; Escudié, Bouyer and Liné, 2004; Delafosse et al., 2008). The results of the average hydrodynamic characteristics presented in Fig. 5.3 and Fig. 5.4 are in agreement with the work of Soos et al. (2013) who examined the flow field at Re~12,000 in a tank equipped with a RT and four tubular baffles positioned at similar $d_{\text{probe}}/D_T \sim 0.36$ (versus $d_{\text{probe}}/D_T \sim 0.34$ in the current work) with $d_{\text{probe}}$ being the distance from the probe centre to the centre of the tank (see Fig. 2.1(d)). In their work it was mentioned that the presence of tubular baffles close to the impeller reduced the magnitude of $u_r$ compared to the standard baffled tank configuration, but increased the width of the discharge stream in proximity to the tubular baffle. Furthermore, in proximity to the baffle they found average kinetic energy, $k \sim 0.1u_{\text{tip}}^2$ but reported that the total turbulence was ~40% lower compared to a standardised baffled vessel (Escudié and Liné, 2003; Soos et al., 2013).
5.2.2 Characterisation of vorticity distribution

The impact of the presence of probes on vorticity distribution was studied by estimating the tangential and axial vorticity magnitude on one vertical (P1) and on three horizontal planes which are presented in Fig. 5.5 and Fig. 5.6, respectively. Contours of the phase resolved tangential vorticity magnitude, \(\langle \xi_\theta \rangle\), are presented in Fig 5.5, as extracted from LES data, for \(\varphi=10^\circ-40^\circ\) for the baffled configuration with B1 and small probes (Pa+B1) and compared to the corresponding vorticity data for the baffled un-probed vessels with B1 and B2 already discussed in chapter 4. From Fig. 5.5 it is evident that the radial propagation of the trailing vortices is rapidly interrupted by the presence of the probes and the vortices elongate vertically along the probe surface and separate from each other. Contrary to the baffled vessels, where vorticity magnitude \(\langle \xi_\theta \rangle\) decreases after \(\varphi=30^\circ\), in Pa+B1 the trailing vortices appear to maintain a higher intensity with increasing blade angle. For example in the B1 and B2 configurations at \(\varphi=30^\circ\), \(\langle \xi_\theta \rangle\)~±15 and ±20 and it decreases to \(\langle \xi_\theta \rangle\)~±5 and ±10, respectively, at \(\varphi=40^\circ\) whereas for the Pa+B1 vessel \(\langle \xi_\theta \rangle\) is always higher than ~±30 across all phase angles. Moreover, in Pa+B1 for all \(\varphi\) the upper vortex appears to be consistently larger than the bottom one as opposed to the baffled un-probed configurations where the upper vortex size appears to be larger up to \(\varphi=20^\circ\) and is then reduced at \(\varphi=30^\circ\) and 40°. In addition, when comparing Fig. 5.4 and Fig. 5.5 for the vessel with Pa+B1, it is evident that the radial position of the maximum \(k'\) is well correlated with the position of the trailing vortices \((r/D_T\sim0.25)\). The results presented in Fig. 5.5 are in good agreement with Soos et al. (2013) who assessed the evolution of trailing vortices in proximity to tubular baffles. In their work, they reported that the radial propagation of the trailing vortices was suppressed due to the interference of the tubular baffles with the impeller discharge stream, leading to their limited expansion compared to a standard fully baffled vessel geometry (Derksen and den Akker, 1999; Escudié and Liné, 2003; Soos et al., 2013).

Contours of the ensemble average axial vorticity magnitude \(\langle \xi_z = D_T \omega_z / u_{tip} \rangle\) combined with average velocity vector plots, are presented in Fig. 5.6 for the horizontal plane corresponding to the impeller centreline \((z/D_T\sim0.35)\), based on LES data, for the four different vessel configurations investigated. For all cases the impeller
is located at the centre (-0.22<r/DT<0.22) and rotates clockwise as indicated by the average velocity vector field. In the UB vessel (Fig. 5.6(a)) the average $\xi_z$ increases in proximity to the impeller to approximately $|\xi_z|\sim2.5$ and it reduces to $\sim0$ away from the impeller blades. In the absence of internals (baffles and/or probes) the velocity vectors follow an undisrupted circumferential motion caused by the impeller rotation. In the baffled vessels (Fig. 5.6(b) and Fig. 5.6(d)) the vorticity magnitude increases to $|\xi_z|\sim3$ and $|\xi_z|\sim3.5$ for B1 and B2, respectively, and reaches maximum values at the corners of the baffle. Moreover, the increase in the resistance of the system is denoted as the intense tangential motion is interrupted and smaller counter-clockwise rotating vortices are formed behind the baffles, increasing in size between B1 and B2. The addition of probes (Fig. 5.6(c)) is further disrupting the circumferential motion of the flow leading to the generation of two symmetrical vortices around the probe. The resulting wakes are linked to a further increase in resistance and drag in the interior of the tank and may explain the rise in the $P_0$ between the B1 and P3+B1 configurations, as observed in Fig. 5.1(a).

The wake generated around the probes was qualitatively and quantitatively investigated in Fig. 5.7 where contours of the ensemble average $\xi_z = D_T\omega_z/u_{tip}$ combined with average velocity vector plots are presented for three horizontal planes across different tank elevations ($z/D_T\sim0.5$, $z/D_T\sim0.35$ and $z/D_T\sim0.22$) based on LES data. The wake, generated by the impeller stream impinging on the probes, is evident in all the different planes presented, but appears to change direction along the probe height. Specifically at the impeller centreline, $z/D_T\sim0.35$, (Fig. 5.7(b)) the flow impingement on the probes occurs in proximity to the blade passage, where the radial velocity component is high, thus leading to the generation of a wake around the probes pointing outwards and towards the vessel wall. For the horizontal planes below ($z/D_T\sim0.22$) and above ($z/D_T\sim0.5$) the impeller (Fig. 5.7(a) and Fig. 5.7(c) respectively) vorticity profiles appear similar. In those areas the intensity of $u_r$ component drops and tangential motion becomes more pronounced, changing the inclination of the wakes formed around the probe, which now appear to be parallel to the vessel walls.
The distribution of vorticity around the probe was examined as an indirect way to obtain an approximate estimate of the shear on the probe walls (i.e., both vorticity and shear are based on velocity gradients (Collignon et al., 2010)). Close-ups of the vorticity are presented in Fig. 5.8 for the O2 probe only, but data are expected to be identical for the pH probe as the bioreactor configuration is symmetrical and the two probes are of same size. In Fig. 5.8 contours of phase resolved axial vorticity magnitude, $\langle \xi_z \rangle = D_T \langle \omega_z \rangle / u_{tip} \ (\varphi=0^\circ, \ 20^\circ \text{ and } 40^\circ)$ are illustrated for three different tank elevations ($z/D_T \approx 0.5, z/D_T \approx 0.35 \text{ and } z/D_T \approx 0.22$) based on LES data. In the last column of Fig. 5.8, the profile of $\langle \xi_z \rangle$ is presented around the probe (an average of vorticity values included in 3 mm around the probe were considered), starting from $\alpha=0^\circ$ and moving clockwise around the probe circumference, as indicated by the black arrow on the plots in the first column. As expected from the ensemble average vorticity shown Fig. 5.7, the distributions of $\langle \xi_z \rangle$ above and below the impeller (Fig. 5.8(a) and (c)) are similar and the wake is illustrated to be parallel to the reactor wall across all the phase angles presented ($\varphi=0^\circ, \ 20^\circ \text{ and } 40^\circ$). In both planes (Fig. 5.8(a) and Fig. 5.8(c)) the resulting vorticity profiles around the probe exhibit two maxima, at $\alpha\approx 140^\circ$ and $230^\circ$, and two minima at $\alpha\approx 30^\circ$ and $320^\circ$ each corresponding to the wake behind the probe and the point of flow impingement on the probe, respectively. For the impeller plane the distribution of $\langle \xi_z \rangle$ appears to change with blade angle (Fig. 5.8(b)). At $\varphi=0^\circ$, where the impeller blade is aligned with the probe, the radial velocity component is higher and the wake is more inclined towards the vessel wall. At $\varphi=20^\circ$ and $40^\circ$ the tangential velocity increases and pushes the direction of the wake downwards. The wake developed around the probe is observed to interact with the impeller trailing vortices, as the blade angle increases, resulting in changes in vorticity profile across the different $\varphi$, a phenomenon which is more pronounced for $\varphi=20^\circ \text{ and } 40^\circ$. Moreover, maximum vorticity values at this elevation ($z/D_T \approx 0.35$) are observed at a similar point around the probe ($\alpha\approx 210^\circ-230^\circ$) which is related to the impeller jet impingement.

5.2.2.3 Characterisation of pressure distribution

To further investigate the impact of the presence of probes on the overall flow field and power requirements, LES was used to extract the distribution of static pressure across all planes of measurement. Contours of the phase resolved pressure
distribution are presented in Fig. 5.9 (φ=0°, 20° and 40°) for three different elevations (z/DT ~0.5, z/DT ~0.35 and z/DT ~0.22). The pressure profile around the probe is presented in the last column of Fig. 5.9, taking into account the average pressure from an area expanding 3 mm around the probe (similarly to Fig. 5.8 for vorticity distribution). For the horizontal planes above and below the impeller (Fig. 5.9(a) and Fig. 5.9(c)), the pressure profiles are similar with minimum and maximum values appearing at α~180° and 280°, respectively. At the impeller centreline (Fig. 5.9(b)) the pressure distribution around the probe appears to depend on the blade angle but its maximum is always positioned at α~220°. The point of the maximum pressure is defined as the stagnation point and corresponds to minimum fluid velocity. Contrary to Fig. 5.9(a) and Fig. 5.9(c), where the minimum pressure is always positioned at α~180°, at the impeller plane the wake around the probe interacts with the impeller jet and trailing vortex core affecting the location of the area of minimum pressure. For example, according to the ⟨p⟩ contour at φ=0° the area of minimum pressure (⟨p⟩~40 Pa) corresponds to the trailing vortex core generated by the approaching blade (illustrated on the left of the contour at φ=0° for z/DT~0.35). This area of pressure minimum moves with the impeller blade and at φ=40° appears to overlap with the probe wake resulting to a ⟨p⟩ minimum at α~150° (⟨p⟩~40 Pa), evident from the pressure profile around the probe and the corresponding contour plot.

5.2.2.4 Estimation of drag

In addition to the use of experimental techniques and empirical correlations, determination of power consumption can be conducted via measuring form (or pressure) and friction (or skin) drag forces of the system as detailed in chapter 2 (Section 2.3.5 eq. 2.5-2.9) (Tay and Tatterson, 1985). In the current work the drag generated by the presence of probes was examined and correlated with the equivalent power draw based on eq. 2.5, according to which FD is the drag force and U∞ is the free stream velocity, i.e. the velocity of the fluid approaching the probe in the current study.

Form and friction drag and the corresponding power were estimated from LES data (using eq. 5.2 and eq. 5.3) by integrating the contributing components from the
pressure and shear stress, \( i \) and \( j \), respectively along the height of each probe. Three horizontal planes were used to extract pressure and velocity data across three different tank elevations (\( z/D_T \sim 0.5 \), \( z/D_T \sim 0.35 \) and \( z/D_T \sim 0.22 \)) and were integrated and considered constant along the corresponding section of the probe \((l_A, l_B \) and \( l_C)\) as indicated in Fig. 5.10 and Table 5.3. For the calculation of power (eq. 2.5), drag was multiplied by the free stream velocity, \( U_\infty \), corresponding to each section of the tank. The results for both form and friction drag are summarised in Table 5.3.

\[
F_{\text{form}} = 2 \cdot \int p(n \cdot i)dA \rightarrow P_{\text{form}} = 2 \cdot \int p \cos(a) \cdot R_{P_a} \cdot l_{A,B,C} \cdot U_\infty \cdot A_{A,B,C} \quad \cdots \cdots \quad (5.2)
\]

\[
F_{\text{friction}} = 2 \cdot \int (\tau \cdot i)dA \rightarrow P_{\text{friction}} = 2 \cdot \int \tau \sin(a) \cdot R_{P_a} \cdot l_{A,B,C} \cdot U_\infty \cdot A_{A,B,C} \quad \cdots \cdots \quad (5.3)
\]

In eq. 5.2 and eq. 5.3, \( \alpha (\degree) \) indicates the circumference of the cylindrical probe, \( R_{P_a} \) is the radius of the probe \( P_a \), \( l_{A,B,C} \) is the length of each section (Fig. 5.10) and \( U_\infty \cdot A_{A,B,C} \) is the free stream velocity corresponding to each section of the vessel \( (A, B \) and \( C) \). Both form and friction drag were multiplied by 2 in order to account for both probes. In eq. 5.3 \( \tau \) is the shear stress computed from velocity gradients in \( r \) and \( \theta \) as follows (eq. 5.4-5.5):

\[
\tau_{rr} = 2\mu \frac{\partial u_r}{\partial \theta} \quad \cdots \cdots \quad \cdots \cdots \quad \cdots \cdots \quad (5.4)
\]

\[
\tau_{\theta r} = \mu \left(\frac{1}{r} \frac{\partial u_r}{\partial \theta} + \frac{\partial u_\theta}{\partial r}\right) \quad \cdots \cdots \quad \cdots \cdots \quad \cdots \cdots \quad (5.5)
\]

According to the results presented in Table 5.3, \( P_{\text{friction}} \) is negligible compared to \( P_{\text{form}} \) but this is expected as the total drag force developed around a bluff body, such as the cylindrical probe in this case, is dominated by the form drag. Tay et al. (1985) followed a similar methodology to estimate the power number of a four-blade PBT by measuring experimentally the pressure drop across the blades and reported a \( P_0 \sim 0.9 \) which was close to the \( P_0 \) value calculated from empirical correlations \((P_0 \sim 1)\). In their work the friction drag was estimated to be two orders of magnitude lower than the form drag, which is in agreement with the results reported in Table 5.3 (Bates, Fondy and Corpstein, 1963; Tay and Tatterson, 1985).

Results of Table 5.3 were compared with the power measured experimentally for the baffled bioreactors with B1 and \( P_a+B1 \) at \( Re=3,732 \) (Table 5.1). To isolate the
contribution to the power increase determined by the presence of the probes, the average $P_0$ difference between the baffled vessel with B1 and $P_a$+B1 was estimated as follows:

$$P[W] = \overline{P_0} \cdot \rho_L \cdot N^3 \cdot D_i^5 \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (5.6)$$

where $\overline{P_0}$~0.33 represents the mean $P_0$ difference between the two vessels (i.e. baffled with B1 and $P_a$+B1). The power estimated from eq. 5.6 was $P$~5.79·$10^{-4}$ W and is in close agreement with the total $P_{form}$ estimated from eq. 5.2 and presented in Table 5.3 (relative error of ~7% calculated based on $100 \times \frac{|P[W]_{\text{experiment}} - P[W]_{\text{form drag}}|}{P[W]_{\text{experiment}}}$), indicating that the form drag created by the probes was responsible for the excess in power between these two vessel configurations and is expected to increase with probe size.

5.2.2.5 Introduction to new probe configuration

To reduce the form drag and therefore the excessive power requirements caused by the presence of cylindrical probes, novel streamlined probes were designed and used to experimentally measure power number in the 1 L Univessel® (Sartorius) equipped with a six-blade FBT (Fig. 2.3). The impeller was similar to that used in the 250 mL custom made bioreactor configuration with the same $D_i/D_T$~0.44 but lower $w_b/D_i$ ($w_b/D_i$~0.16 in the 1 L Univessel® versus $w_b/D_i$~0.26 for the 250 mL custom made vessel). Two types of symmetric streamlined probes were used with maximum thickness ($\delta$) equal to a cylindrical probe, zero camber and varying chord length ($l$): $P_{str,1}$ with $\delta/l$=0.3 and $V(\%)$~1 and a custom made teardrop probe, $P_{str,2}$, with $\delta/l$=0.54 and $V(\%)$~0.5. Details on the probe sizes are presented in Fig. 2.3 (chapter 2).

Measured $P_0$ in the turbulent regime for $P_{str,1}$ and $P_{str,2}$ are presented in Fig. 5.11 and compared with $P_0$ in the UB/NI case and in the presence of a single cylindrical probe with volume blockage ratio ($V(\%)$) ~0.2. Both streamlined probes are positioned at 0° with respect to $y$ axis (i.e. parallel to the vessel wall). As expected the lowest $P_0$ is measured for the UB/NI vessel and it continuously decreases with increasing Re. With the addition of probes the power number increases and appears to reach a plateau in the presence of $P_{str,2}$ and the cylindrical probe. It is interesting to note that although
\( P_{str,1} \) has the highest volume blockage ratio (~1%), its corresponding \( P_0 \) is lower compared to the cylindrical probe with \( V(\%) \sim 0.2 \) and \( P_{str,2} \) with \( V(\%) \sim 0.5 \). The latter probes lead to similar \( P_0 \sim 1.18 \) which is 18% higher than that measured for \( P_{str,1} \) (\( P_0 \sim 1 \)) but this can be attributed to their higher \( \delta/l \) ratios which lead to an increase in form drag and therefore greater \( P_0 \) (Gudmundsson, 2014).

The impact of the angle of probe orientation (\( \gamma \)) on \( P_0 \) was examined and the results are presented in Fig. 5.12, where \( P_0 \) was measured across different inclinations of the streamlined probes and compared with the \( P_0 \) in the absence of internals and in the presence of one cylindrical probe with \( V(\%) \sim 0.2 \). For \( P_{str,1} \) (Fig. 5.12(a)) the lowest \( P_0 \) is observed at \( \gamma = 0^\circ \) and incrementally increases with increasing \( \gamma \). For example, moving from \( \gamma = 0^\circ \) to \( \gamma = 15^\circ \) the \( P_0 \) rises by approximately 26% and it increases further by ~34% and 15% at \( \gamma = 30^\circ \) (\( P_0 \sim 1.75 \)) and \( 45^\circ \) (\( P_0 \sim 2 \)) respectively. Interestingly, when \( \gamma > 0^\circ \) the resulting \( P_0 \) is higher than the corresponding one measured in the presence of a cylindrical probe. Specifically, \( P_0 \) rises ~12% when switching between the cylindrical probe (\( P_0 \sim 1.18 \)) and the \( P_{str,1} \) at \( \gamma = 15^\circ \) (\( P_0 \sim 1.31 \)), which is the lowest \( \gamma \) used after \( 0^\circ \). In the case of \( P_{str,1} \) the largest percentage increase of \( P_0 \) is observed between \( \gamma = 15^\circ \) and \( 30^\circ \) (~34% increase). This rise of \( P_0 \) can be attributed to the fact that by increasing the probe inclination (with respect to \( y \) axis, as indicated by the red dotted line in Fig. 5.12), the aerodynamic probe starts to expose its longest side to the incoming flow, therefore perturbing more the flow and acting as a large baffle (with width \( l/D_T \sim 0.18 \)). At \( \gamma = 45^\circ \) the trailing edge is in close proximity to the reactor wall, intensifying the baffle effect which leads to an increase in flow resistance and eventually power.

For the \( P_{str,2} \), \( P_0 \) results are presented in Fig. 5.12(b). Similarly to \( P_{str,1} \) the lowest \( P_0 \) values are observed at \( \gamma = 0^\circ \) (\( P_0 \sim 1.17 \)) but at higher \( \gamma \), \( P_0 \) increases at a lower rate compared to \( P_{str,1} \). For example, \( P_0 \) rises ~2.5% from \( \gamma = 0^\circ \) to \( 15^\circ \) but when \( 30^\circ < \gamma < 60^\circ \) the corresponding increase in power number equals ~10% for each 15° rise of the angle denoting the probe orientation \( \gamma \). At the highest \( \gamma \) (\( \gamma = 60^\circ \)), \( P_0 \) measured with \( P_{str,2} \) is lower than \( P_0 \) measured with \( P_{str,1} \) when \( \gamma = 45^\circ \) (\( P_0 \sim 1.6 \) for \( P_{str,2} \) versus \( P_0 \sim 2 \) for \( P_{str,1} \)) indicating that the baffle effect in the case of \( P_{str,1} \) is more pronounced, which can be
also explained by the highest $l/D_T$ of $P_{str,1}$ compared to $P_{str,2}$ ($l/D_T=0.18$ for $P_{str,1}$ versus $l/D_T\approx0.1$ for $P_{str,2}$).

Considering that $P_0$ is a function of the probe geometry and orientation as demonstrated in Fig. 5.11 and Fig. 5.12 the use of streamlined probes can be beneficial for the reduction of power requirements but results presented in this work highlight that the probe design should be conducted with care as an optimal configuration depends on various parameters, including size, $\delta/l$, and local airfoil inclination with respect to the incoming flow. The dependence of form drag and therefore $P_0$ on the probe orientation can be attributed to the fluid flow in proximity to the probe and specifically to the boundary layer formed. At low $\gamma$, the boundary layers remain attached on either side of the streamlined probe, along almost the entire chord length. However, increasing $\gamma$ will intensify the adverse pressure gradients around the probe leading to an increase in form drag and consequently $P_0$. At very high probe inclination (and $\gamma\approx90^\circ$), and assuming that the probe will not be in contact with the vessel wall (baffle effect), the form drag would reach the highest value and the streamlined probe would act in a similar way to a bluff body, significantly increasing $P_0$ (Gudmundsson, 2014). Interestingly, considering that the wake developed around the probe changes direction along the tank height (according to Fig. 5.7) an ideal probe configuration would change shape and inclination depending on the observed flow patterns in each area of the tank.

5.3 Summary

In this chapter characterisation of power requirements in SDMs with different working volumes and configurations was presented. The impact of baffles and probes on $P_0$ was analysed for both axial and radial flow impellers (using PBT and FBT respectively) across the entire range of $Re$ and the flow in the 250 mL custom made radially agitated vessel equipped with both baffles and probes (Fig. 2.1(d)) was examined via CFD. Extracted velocity and pressure data were used to qualitatively and quantitatively characterise vorticity and pressure distributions at different tank elevations, focusing on the estimation of form and friction drag developed due to the presence of probes. Flow field results were used for the design of novel probe
configurations aiming to reduce the excessive form drag created by the standard cylindrical probes, which led to a reduction of the corresponding power requirements.

The isolated impact of baffles and probes on $P_0$ was investigated for three commercial small scale bioreactors with working volumes 60 mL – 1 L and showed that the presence of probes led to a rise of turbulent $P_0$ equivalent to that produced by baffles. Results at high $Re$ indicated that $P_0$ became greater with the addition of internals with a tendency to flatten with increasing $V(\%)$. Measurements for radially and axially agitated vessels indicated that the presence of internals (i.e. probes and/or baffles) had a greater impact on flow in radial impellers, causing higher percentage increase of $P_0$ compared to axial impellers, while it was found that baffle contribution to the power input was significantly higher than that associated with the probe presence.

The impact of probes on hydrodynamics was studied for the 250 mL custom made bioreactor equipped with a FBT, three equally spaced baffles (B1) and two cylindrical probes ($P_a$), $P_a+B1$ at $Re=3,732$. Results from CFD LES showed a ~15% and ~20% increase in velocity magnitude and turbulent kinetic energy respectively between the B1 and $P_a+B1$ configurations. Comparison between $P_a+B1$ and B2 (un-probed) vessels exhibited a further increase in velocity and turbulence levels underlining the high impact of baffle presence and size on flow.

The impact of probes on vorticity levels was investigated across different tank elevations. The axial component of vorticity magnitude indicated the presence of a wake developed around the cylindrical probes, which changed direction along their heights depending on the prevailing radial or tangential flow motion. Vorticity profiles below and above the impeller plane had similar distributions across the different phase angles while at the impeller centreline the interaction between the probe wake and impeller trailing vortices was evident. The corresponding pressure distribution around the probes was analysed and data were used for the estimation of form drag which appeared to be equivalent to the $P_0$ increase due to the presence of probes.

Two novel streamlined probes were designed and the resulting $P_0$ was compared with the one obtained with standardised cylindrical configurations. Data showed that the
resulting power was a function of the size (i.e. aspect ratio) \((\delta/l)\) and inclination of the streamlined probes. Lower \(\delta/l\) resulted in a more streamlined body decreasing the form drag hence the \(P_0\). Orientation of the probes was inherently related to the angle of the fluid approaching and showed that the lowest values of \(P_0\) were obtained when the streamlined probes were parallel to the vessel wall. Increasing the probe inclination (angle \(\gamma\)) led to higher values of \(P_0\) due to rise in form drag and at the highest \(\gamma\) (\(\gamma=45^\circ\) and \(60^\circ\) for \(P_{\text{str,1}}\) and \(P_{\text{str,2}}\) respectively) \(P_0\) was the maximum due to the probe resembling a baffle (baffle effect).

The analysis presented in the current chapter underlines the impact of bioreactor internals on the flow and power consumption. It is suggested that optimisation of internal design results in reduction of the overall resistance of the system, decreasing turbulence levels and power requirements. The measured power number in small scale reactors appears to increase in the presence of internals with significant deviations from well-established empirical data, or even \(P_0\) values provided by equipment vendors, depending on impeller type and flow pattern. In the current work, \(P_0\) was found to increase \(~30\%\) and \(~10\%-16\%\) for the FBT and the PBT respectively, in the presence of both probes and baffles compared to cited literature referring to standardised tanks with volumes 10-50 L (Bates, Fondy and Corpstein, 1963; Chapple et al., 2002). The above observations apply even for commercial SDMs as comparison of the experimentally measured \(P_0\) when all internals are present with information provided by equipment vendors, might be different with the latter being slightly lower as companies provide a single power number value, usually corresponding to a baffled configuration of a specific small scale vessel (unpublished data). When scaling-down based on constant \(P/V\), those discrepancies in \(P_0\) between small and large vessel volumes and differences among various configurations of a specific SDM, will lead to variations in local energy dissipation rate resulting in dissimilarities in the reactor physical environment across scales and affecting the flow patterns and mixing performance. Those disparities are expected to grow as reactor scales become smaller and highlight that the detailed engineering characterisation of small scale vessels is essential for scaling optimisation and the development of efficient SDMs operating equivalently to the larger scales.
Figure 5.1: Experimental power number ($P_0$) curves for SDMs equipped with baffles and/or probes agitated with radial (FBT) and axial impellers (PBT and 3BS impeller): (a) 250 mL custom made bioreactor equipped with a FBT, (b) 60 mL EasyMax™ (Mettler-Toledo) bioreactor equipped with a PBT (up-pumping mode, experiments conducted by Dr Anne de Lamotte) and (c) 1 L Univessel® (Sartorius) bioreactor equipped with a 3BS impeller (up-pumping mode) (experiments conducted by Tom Wyrobnik (Wyrobnik et al., 2021)).
Table 5.1: Summary of $P_0$ values measured experimentally and estimated computationally via CFD LES at $Re=3,732$, for all configurations studied in the custom made 250 mL bioreactor equipped with a FBT.

<table>
<thead>
<tr>
<th>Vessel Configuration (FBT)</th>
<th>$P_0[-]_{\text{exp}}$</th>
<th>$P_0[-]_{\text{LES}}$</th>
<th>$\overline{P_{0_{\text{exp, LES}}}}$</th>
<th>Relative error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB</td>
<td>1.9</td>
<td>2.5</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>B1</td>
<td>4.6</td>
<td>4.8</td>
<td>4.7</td>
<td>4</td>
</tr>
<tr>
<td>B2</td>
<td>5.2</td>
<td>5.5</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>$P_a$</td>
<td>3.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_b$</td>
<td>4.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_a+B1$</td>
<td>5.02</td>
<td>5.05</td>
<td>5.035</td>
<td>0.6</td>
</tr>
<tr>
<td>$P_b+B1$</td>
<td>5.15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5.2: Summary of the experimental $P_0$ results at the fully turbulent regime ($Re>5,000$) for the FBT and 45° PBT.

<table>
<thead>
<tr>
<th>Vessel Configuration (FBT)</th>
<th>$P_0 [\text{-}]exp$</th>
<th>Vessel Configuration (PBT)</th>
<th>$P_0 [\text{-}]exp$</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB</td>
<td>1.5</td>
<td>UB</td>
<td>0.5</td>
</tr>
<tr>
<td>B1</td>
<td>5.1</td>
<td>B</td>
<td>1.4</td>
</tr>
<tr>
<td>B2</td>
<td>5.3</td>
<td>T probe (V(%)~2.3)</td>
<td>0.8</td>
</tr>
<tr>
<td>$P_a$ (V(%)~2.8)</td>
<td>3.8</td>
<td>pH probe (V(%)~5.7)</td>
<td>1.12</td>
</tr>
<tr>
<td>$P_b$ (V(%)~6.3)</td>
<td>4.5</td>
<td>T+pH probes (V(%)~8)</td>
<td>1.2</td>
</tr>
<tr>
<td>$P_a$+B1</td>
<td>6</td>
<td>T+pH probes+L1+L2 (V(%)~8.4)</td>
<td>1.32</td>
</tr>
<tr>
<td>$P_b$+B1</td>
<td>6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bates et al. (1963)</td>
<td>5</td>
<td>Chapple et al. (2002)</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Figure 5.2: Impact of probes and baffles on the experimentally measured $P_0$ for Re>5,000: ((a), (b)) for the 250 mL custom made bioreactor equipped with a FBT; ((c) and (f)) for the 60 mL EasyMax™ (Mettler-Toledo) equipped with a PBT (experiments conducted by Dr Anne de Lamotte).
Figure 5.3: Impact of baffle and probes on velocity magnitude ($|u|/u_{tip}$) based on LES data for the vertical planes $P_1$ and $P_2$. 
Figure 5.4: Impact of baffle and probes on turbulent kinetic energy ($k'/u_{tip}^2$) based on LES data for the vertical planes $P_1$ and $P_2$. 

Unbaffled (UB)  \[ \text{B1} \]  \[ P_a + \text{B1} \]  \[ \text{B2} \]
Figure 5.5: Contour plots of phase resolved tangential vorticity magnitude $(\langle \xi_\theta \rangle = D_T (\omega_\theta / u_{tip})$) for the vertical plane $P_1$ based on three different configurations studied including baffled with B1, baffled with B1 and small probes $P_a$ ($P_a + B1$) and baffled vessel with B2. Results correspond to LES data for $\varphi = 10^\circ, 20^\circ, 30^\circ$ and $40^\circ$. 
Figure 5.6: Contour plots of ensemble average axial vorticity magnitude ($\xi_z = D_T \omega_z / u_{tip}$) for the horizontal plane at the impeller centreline ($z/D_T \sim 0.35$) for four different configurations studied of the 250 mL custom made bioreactor: (a) UB, (b) baffled with B1, (c) baffled with B1 and small probes $P_a (P_a+B1)$ and (d) baffled vessel with B2. Results correspond to LES data.
Figure 5.7: Contour plots of ensemble average tangential vorticity magnitude ($\xi_z = D_T \omega_z / u_{tip}$) for three horizontal planes at three different elevations in the 250 mL custom made bioreactor configuration equipped with baffles B1 and probes $P_a$ ($P_a+B1$) and a FBT: (a) horizontal plane above the impeller ($z/D_T \sim 0.5$), (b) horizontal plane at the impeller centreline ($z/D_T \sim 0.35$) and (c) horizontal plane below the impeller ($z/D_T \sim 0.22$). The different elevations of the horizontal planes investigated are indicated with the red dotted line on the bioreactor schematic. Results presented are based on LES dataset.
Figure 5.8: Phase resolved ($\phi = 0^\circ, 20^\circ, 40^\circ$) vorticity ($\langle \zeta_z \rangle = D_T (\omega_z) \cdot \frac{u_{tip}}{u}$) distribution around an O$_2$ probe in the 250 mL custom made bioreactor configuration equipped with baffles B1 and probes P$_a$ (P$_a$+B1) and a FBT. Results correspond to three different plane elevations (a) above impeller ($z/D_T \sim 0.5$), (b) impeller centreline ($z/D_T \sim 0.35$) and (c) below impeller ($z/D_T \sim 0.22$). The different elevations of the horizontal planes investigated are indicated with the red dotted line on the right hand side. The last column illustrates the distribution of $\langle \zeta_z \rangle$ around the probe for $\phi = 0^\circ$ (black line), $\phi = 20^\circ$ (blue line) and $\phi = 40^\circ$ (red line). Results correspond to LES data.
Figure 5.9: Phase resolved ($\phi = 0^\circ, 20^\circ, 40^\circ$) pressure ($\langle p \rangle$ [Pascals]) distribution around an O$_2$ probe in the 250 mL custom made bioreactor configuration equipped with baffles B1 and probes P$_a$ (P$_a$+B1) and a FBT. Results correspond to three different plane elevations (a) above impeller ($z/D_T \sim 0.5$), (b) impeller centreline ($z/D_T \sim 0.35$) and (c) below impeller ($z/D_T \sim 0.22$). The different elevations of the horizontal planes investigated are indicated with the red dotted line on the right hand side. The last column illustrates the distribution of $\langle p \rangle$[Pascals] around the probe for $\phi = 0^\circ$ (black line), $\phi = 20^\circ$ (blue line) and $\phi = 40^\circ$ (red line). Results correspond to LES data.
Table 5.3: Summary of the power [W] estimated from form (pressure) and friction drag along the height of the 250 mL custom made bioreactor probes (two probes, \(P_a\)). Results are based on pressure and velocity data extracted from LES.

<table>
<thead>
<tr>
<th>Power from Drag [W]/Corresponding part on probes</th>
<th>Power from Form Drag [W]</th>
<th>Power from Friction Drag [W]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section A</td>
<td>2.3 ( \times ) 10(^{-5})</td>
<td>3.8 ( \times ) 10(^{-6})</td>
</tr>
<tr>
<td>Section B</td>
<td>4.2 ( \times ) 10(^{-4})</td>
<td>4 ( \times ) 10(^{-6})</td>
</tr>
<tr>
<td>Section C</td>
<td>9.3 ( \times ) 10(^{-5})</td>
<td>5.3 ( \times ) 10(^{-8})</td>
</tr>
<tr>
<td>Total</td>
<td>5.4 ( \times ) 10(^{-4})</td>
<td>7.8 ( \times ) 10(^{-6})</td>
</tr>
</tbody>
</table>

Figure 5.10: Visualisation of the three sections of the tank that were integrated for the estimation of form and friction drag. The corresponding power from drag forces are summarised in Table 5.3 for each section.
Figure 5.11: Impact of probe presence and shape on $P_0$ for 1 L Univessel® (Sartorius) bioreactor equipped with a FBT. $P_0$ is illustrated based on experimental measurements conducted in the turbulent regime for the UB/NI case, a cylindrical probe, and two symmetric streamlined probes ($P_{str,1}$, $P_{str,2}$) with equal thickness and different chord length. Results correspond to $0^\circ$ rotation of the streamlined probes i.e. probes are parallel to the vessel wall.
Figure 5.12: Impact of the angle of probe orientation ($\gamma$) on $P_0$ for (a) streamlined probe 1, $P_{str,1}$, and (b) streamlined probe 2, $P_{str,2}$, in the 1 L Univessel® (Sartorius) bioreactor equipped with a FBT. Maximum $\gamma$ reached corresponded to $\gamma = 45^\circ$ for (a) and $\gamma = 60^\circ$ for (b) with respect to $y$ axis. Results correspond to experimental measurements conducted in the turbulent regime.
Chapter 6: Conclusions and recommendations for future work

6.1 Main findings of the investigation

In this work, a systematic engineering characterisation in a scale-down model (250 mL), equipped with a FBT, was performed focusing on the impact of the bioreactor geometric features on the hydrodynamics and power consumption. Firstly, the impact of baffle presence and size on the average velocity field and turbulence levels was assessed and the predictions of CFD simulations (URANS and LES) was validated against experimental PIV data. For this study three different bioreactor configurations were used: one unbaffled (UB) and two baffled configurations with baffles of increasing size. The agreement between LES and PIV is excellent for both time and phase resolved data across all the vessel configurations studied. In URANS simulations, the average velocity components are satisfactorily predicted for the baffled bioreactors (Fig. 3.4-3.5 and Fig. 3.7-3.12), but the model fails to reproduce the mean velocity field for the UB case, both in terms of magnitude and spatial distribution (Fig. 3.3).

A study on the accuracy of model predictions for the mean tangential velocity component by different CFD approaches was carried out and indicated that, for the baffled vessel with B1, LES is in agreement with experiments at the impeller centreline and a relative error of 10-25% was estimated for the regions above and below the impeller (Fig. 3.6). In URANS simulations, even though \( u_\theta \) is in agreement with LES for velocities at the impeller level, in the regions above and below the model overestimated tangential velocity for the UB vessel by ~30% compared to LES. Those discrepancies dropped to 20% in the presence of smaller baffles (B1) and were insignificant with the addition of larger baffles in the system (B2). These results underline the sensitivity of RANS approach to fully resolve the entire flow field in a closed UB vessel, where the deformation of the free surface is suppressed and justifies the discrepancies with respect to axial and radial velocity components. In the baffled vessels, \( u_\theta \) peaks at the impeller area and is higher in the UB case while a 10% reduction is observed with baffle presence and size. Tangential velocity profile and non-zero values above and below the impeller centreline confirm the importance of baffles in the elimination of solid body rotation highlighting the impact of baffle size on its reduction and predictions of CFD modelling.
Periodic and turbulent kinetic energy were derived from simulations and experimental data for the three bioreactor configurations and the impact of baffles on turbulence levels were assessed. LES predictions were in agreement with experimental data for both periodic and random contributions (Fig. 3.13-3.15). URANS simulations failed to predict periodic kinetic energy, which can be attributed to the averaging nature of those models that filters out the periodic fluctuations, and consequently do not account for prediction of trailing vortices (Delafosse et al., 2008). Estimations of $k'$ based on isotropic 2D and 3D assumptions showed very good agreement between experiments and simulations, with the latter having slightly lower magnitude. Evaluation of the turbulent fluctuations in the $\theta$ direction was conducted based on the LES dataset and showed that the tangential fluctuating velocity component was always overestimated by the 2D prediction (Fig. 3.16). Nevertheless, profiles of turbulent kinetic energy distribution considering all the velocity components led to better qualitative representation of the characteristic double peak corresponding to trailing vortex formation.

Regarding the impact of baffles on turbulence levels, a two-fold increase in periodic kinetic energy and a three-fold increase in $k'$ is observed between UB and baffled configurations which is attributed to intensification in the flow resistance caused by a rise in drag that is induced by the baffle presence and is amplified with baffle size (Fig. 3.15). The lower levels of turbulence due to periodic fluctuations implied lower periodic stability of the trailing vortices which could be translated to an increase in the random/fluctuating component.

The impact of reactor geometry and impeller type on the development of trailing vortices and generation of flow instabilities was studied for the 250 mL custom made SDM in the presence of two different impeller types, a FBT and a standard RT. Firstly, characterisation of trailing vortices for the FBT case was implemented based on the magnitude of tangential vorticity estimated from LES and PIV data for both UB and baffled configurations. Phase resolved data indicated that in the UB case trailing vortices were distinctive, had equal magnitude and moved radially outwards and in parallel as the blade angle increased. In the presence of baffles the parallel movement of the vortices became weaker due to an impeller jet inclination which increased with baffle size (Fig. 4.1). The intensity of the vortices was also calculated based on average vorticity values which was denoted by a gradual amplification with increasing baffle size (Fig. 4.5).

The stability of trailing vortices was assessed by measuring the angle of the velocity stream according to the ratio of axial and radial velocity components. In the UB tank, the angle of the impeller jet was characterised by minimal fluctuations, but the weakened parallel vortex
motion in the presence of baffles led to periodic oscillations of the angle of the impeller jet, manifested with a frequency of 37-47 revolutions \( (f \sim 0.087-0.11 \text{ Hz}) \) and 57-68 revolutions \( (f \sim 0.06-0.073 \text{ Hz}) \) for the B1 and B2 configurations, respectively. These frequencies could be resolved with PIV experimental data which were long enough to fully capture the instability time scale (Fig. 4.6-4.7).

To elucidate instabilities affecting trailing vortex formation, spatio-temporal velocity data were processed with POD to find principal modes of variation along with FFT to assign the most prominent frequencies to each mode. Flow decomposition revealed three distinct frequencies associated with the flow stream interacting between the tank wall and impeller flow region (Fig. 4.10). These frequencies correspond to the second most energetic modes and were related to similar flow disturbance effects on the mean velocity flow field. The impact of these periodic flow structures on the mean flow was studied by a LOM comprising only the most energetic modes and indicated the presence of a jet instability which was amplified in baffled systems, and was associated with a sharp flow stream emanating from the wall and interfering with the impeller flow region (Fig. 4.12-4.15).

The impact of impeller disk on the stability of the trailing vortices, developed in the baffled 250 mL custom made SDM with B1, was assessed by comparing the flow patterns between a FBT and a standard RT. Phase resolved data showed that tangential vorticity magnitude increased two-fold when the RT was used while both trailing vortices were clearly represented and moved in parallel across all the phase angles \( (\varphi = 0^\circ - 50^\circ) \) (Fig. 4.19). Flow decomposition in the presence of the RT showed that the mode associated with the jet instability was of weaker intensity, had lower energy content (~4% versus ~5% of the total energy for RT and FBT respectively) and induced a jet inclination of low amplitude \( (|\beta| \sim 5^\circ-8^\circ \text{ in the RT versus } |\beta| \sim 15^\circ-17^\circ \text{ in the FBT}) \) which was localised at higher radial distances with minimal interference with the impeller trailing vortices. The corresponding LOM of the most energetic modes associated with this instability induced jet fluctuations of low intensity and revealed the existence of three elements in proximity to the baffles rotating clockwise across different time points (Fig. 4.25-4.26).

Flow decomposition and frequency analysis revealed the existence of modes associated with the presence of baffles which can be sources of macroinstabilities impacting the periodic flow patterns, the energy of which depends on the impeller type and vessel configuration (baffled/unbaffled). Results indicated that LES can be reliably used for the characterisation of the spatial distribution of such instabilities and give an accurate approximation of the energy.
content of the relevant POD modes linked to the baffled presence. Nevertheless, more impeller revolutions are required to accurately resolve the temporal characteristics of such instabilities with computational tools and specify the exact ranges of the relevant frequencies.

The impact of bioreactor internals, including probes and baffles, on power consumption in small scale vessels was investigated for axial and radial flow patterns across three commercial SDMs for high $Re$ ($Re>5,000$). The isolated impact of probes and baffles on turbulent $P_0$ was further assessed for the 250 mL custom made bioreactor and a 60 mL vessel equipped with a FTB and a 45° PBT, respectively. Power number results indicated that the presence of internals had a greater effect on the radial flow and led to higher percentage increase of $P_0$ compared to the axial flow. The probe influence on $P_0$ was analysed as a function of their blockage ratio defined as the volume of the probe over the working volume of the liquid (V(%)). Data showed that the first probe addition for the FBT and PBT increased the $P_0$ ~2.5 and 1.6 times, respectively, compared to the corresponding UB scenario, while overall for the radially agitated vessel a lower V(%) led to higher percentage increase of $P_0$ compared to the axial flow. Similarly transition from a tank with probes and/or addition lines to a vessel with baffles impacted more the $P_0$ of the radial impeller, resulting in a ~25% increased $P_0$ compared to ~6% increase for the axial flow. The combined impact of baffles and probes on $P_0$ was investigated for the FBT and indicated a ~20%-30% increase in $P_0$ depending on the size of the probes used (Fig. 5.2 and Table 5.2).

The impact of probes on hydrodynamics was studied for the 250 mL custom made bioreactor equipped with a FBT, three equally spaced baffles (B1) and two cylindrical probes ($P_a$), $P_a$+B1 at $Re=3,732$. Results from CFD LES showed a ~15-20% increase in velocity magnitude and $k'$ respectively between the B1 and $P_a$+B1 configurations. Comparison between $P_a$+B1 and B2 (no-probes) vessels exhibited a further increase in velocity and turbulence levels underlining the high impact of baffle presence and size on flow. Those results were qualitatively consistent with the corresponding power number data measured by the experiments and predicted by LES at this rotational speed ($N=250$ rpm, $Re=3,732$), demonstrating that at the working $Re$ higher $P_0$ was exhibited by B2 configuration and power number and $k'$ exhibited the same trend (Fig. 5.3-5.4 and Table 5.1).

The impact of probes on vorticity and pressure distribution was investigated in three different tank elevations: above the impeller centreline, at the impeller centreline and below the impeller centreline. Qualitative representation of the wake formed behind the cylindrical probe was similar in the regions above and below the impeller and pointed downwards,
parallel to the vessel wall whereas, at the impeller centreline, intense radial flow generated by
the blade passage impacted the wake direction pushing it towards the vessel wall (Fig. 5.7-5.8). Both pressure and vorticity profiles around the probe showed similar distributions for
the regions above and below the impeller centreline, with maximum and minimum values
being indicated at the same points around the probe circumference which remained
unaffected by the impeller phase angle. At the impeller centreline maximum values for the
pressure profile were predicted at \( \alpha \sim 220^\circ \) though alteration of the position of the maximum
values at the different elevations demonstrated variation of the flow impingement depending
on the height of the tank (Fig. 5.9). Simulation data were used for the estimation of form and
friction drag. Results showed that the calculated form drag was two orders of magnitude
higher than the friction drag, as expected for a bluff body such as the cylindrical probe in the
STR. Form drag was used for the evaluation of power consumption and data demonstrated a
\( P_0 \) increase between the B1 and \( P_a+B1 \) configurations, equivalent to an excess in form drag
caused by the presence of probes (Table 5.3).

Pressure and vorticity distributions were used for the design of two novel probe
configurations with a streamlined shape. \( P_0 \) measurements indicated that the resulting power
was a function of the aspect ratio and the inclination of the streamlined probes. Lower aspect
ratio resulted in a more streamlined body leading to a reduction in form drag, hence \( P_0 \) (Fig.
5.11). However, increasing probe inclination led to higher values of \( P_0 \) due to rise in form drag
and at the highest probe inclination (\( \gamma = 45^\circ \) and \( 60^\circ \) for \( P_{str,1} \) and \( P_{str,2} \) respectively) \( P_0 \) was the
maximum due to the probe resembling a baffle (baffle effect) (Fig. 5.12).

The aforementioned analysis, underlines the impact of bioreactor internals on flow and power
consumption characteristics and suggests that the optimisation of internal design might result
in reduction on the system resistance, decreasing turbulence and power requirements. The
measured power number in small scale reactors appeared to increase in the presence of
internals and deviated significantly from published data corresponding to standardised
vessels (\( V \sim 10-50 \) L), depending on the flow pattern, i.e. \( \sim 30\% \) and \( 10-16\% \) increase for radial
and axial flow, respectively. These results highlight that the size of internals relative to the
reactor volume in SDMs have important implications on the power consumption and should
be thoroughly examined as the reliance on existing data may lead to underestimation of \( P_0 \)
and consequently the dissipative power across scales. Those disparities are expected to grow
as reactor scales become smaller highlighting that the thorough understanding of the
engineering characteristics is essential for the development of robust SDMs qualified to mimic biological operations and optimise bioprocessing.

6.2 Recommendations for future work

Chapter 3 described the development of a validated CFD approach, which can be used to predict the flow field in single phase flow in a baffled and an unbaffled SDM operating at intermediate $Re$ ($Re=3,732$). Limitations of different CFD methodologies (LES versus s URANS) were discussed depending on the vessel geometry (baffled versus unbaffled) and compared to PIV data in proximity to the impeller blade. The introduction of a second gas phase would be beneficial in order for a two-phase validated model to be built, able to quantify and visualise velocity and turbulence characteristics in multiphase flow. Considering the combined high spatial and temporal resolution which can be achieved through CFD simulations, the validated model can be then used to assess the impact of different sparger types and aeration methodologies on hydrodynamics and resulting oxygen levels, completing the optimisation of small scale vessels and giving a deeper insight on understanding bioreactor performance.

In addition, considering that the SDM under investigation has been actively used for antibody producing CHO cell cultures in perfusion mode, the investigation of the impact of cell recirculation on reactor hydrodynamics would shed light to the optimisation of SDMs of comparable working volumes for similar applications. In previous work by Tregidgo et al. (2021) the implications of recirculation loop position and flowrate on mixing time were studied (Tregidgo, 2021). Nevertheless, considering that in most of the cases space constraints in SDMs might limit the ports available for the cell return (Martens, van den End and Streefland, 2013), understanding of the flow environment will provide additional information regarding potential interactions between cell recirculation and flow developed in the bulk of the tank, contributing to the reduction of potential impact of shear on cells.

Moreover, to gain a better understanding of the way reactor flow patterns and hydrodynamics might influence the bioprocess performance, the validated model for the flow of this SDM could be coupled with cell metabolic models in order to establish an end-to-end approach between biological processes and engineering characterisation. Such approach would aim to couple mixing dynamics with cell metabolism to assist in the optimisation of efficient recirculation of nutrients (i.e. elimination of dead zones and implementation of flow homogeneity) in the interior of the vessel, allowing the investigation of novel impeller
configurations and the understanding of process bottlenecks for development of optimised
STRs.

The detailed investigation of flow instabilities related to baffle presence, size and impeller
type in SDMs highlights the sensitivity of bioreactors of smaller volumes in critical design
parameters. Based on those findings stability of flow patterns and frequency analysis would
be beneficial to be conducted at higher rotational speeds and for a range of different volumes,
such as 15 mL - 1 L (used in scale-down reactors), to analyse the relationship between flow
instabilities with the operating Re and working volume.

Considering the current findings regarding the impact of baffle presence and size on velocity
magnitude, turbulence levels, flow instabilities and power consumption, presented in
chapters 3-5 it would be useful to investigate the aforementioned aspects in other tank
configurations. For example, is has been discussed that in squared tanks the corners of the
vessel might behave in a way equivalent to baffles (Li, 2019; Fan et al., 2021), therefore the
impact of vessel corners could be analysed in order to get a better understanding regarding
the correlation between baffled and squared-prismatic vessels.

A thorough study of the influence of bioreactor internals on power consumption and flow in
conjunction with an introduction on optimisation of probe geometry, was presented in
chapter 5. The discrepancies in power number values among SDMs and standardised vessels
and the dependence of \( P_0 \) on the number and the size of internals underlined the need to
investigate power characteristics, flow dynamics and mixing efficiency in vessels of smaller
scales (i.e. in the range of 15 mL- 250 mL) when all internals are present. Given that the
disparities between small and larger scales are expected to grow as the reactor scale becomes
smaller (Nienow, Rielly, et al., 2013b), the impact of probes and/or baffles on power and flow
characteristics in SDMs should be elucidated to facilitate the development of robust SDMs
able to adequately mimic large scale processes. The thorough engineering investigation is
critical in both commercial and custom made small scale vessels, in order to understand how
scaling and flow parameters might differ across different working volumes and develop
optimised SDMs. Moreover, taking into account indications that changes in probe
configurations will lead to reduction in power requirements, further investigation of novel
probe geometries is essential to clarify parameters affecting their optimal performance on
fluid mechanics. In this respect, hydrodynamic and mixing studies, will shed light on the
implications of novel probe configurations on the resulting flow field contributing to further
optimisation of their geometry.
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