

Computational Analysis of Mixing Performance and Hydrodynamic Stress in a 30,000 L Bioreactor

By Emmanuel Yu, Xian Wu, and Chi-Ming Yu

Abstract

Computational fluid dynamics (CFD) was used to evaluate how impeller type and vertical arrangement influence mixing performance and hydrodynamic stress in a nominal 30,000 L stirred-tank bioreactor operated at a 20,000 L working volume. Eight configurations containing different combinations of pitched-blade and Smith turbine impellers were simulated in COMSOL Multiphysics (v6.2) using the standard $k-\varepsilon$ turbulence model. Mixing time, flow patterns, shear stress, and energy dissipation rate (EDR) were compared, and mixing-time mesh sensitivity was assessed using three mesh resolutions. Configurations with more pitched-blade impellers generally produced

stronger axial circulation, shorter mixing times, and higher volume-average shear stress and EDR. The all-pitched-blade configuration mixed fastest, whereas the all-Smith configuration mixed slowest. Local maximum shear stress and EDR depended more strongly on impeller arrangement, and not predicted by vessel-average behavior alone. Comparison with a 3,000 L small-scale vessel showed that matching the selected impeller configuration and power input per unit volume (P/V) did not provide hydrodynamic equivalence. These results highlight the importance of evaluating impeller type, placement, and vessel scale together when balancing mixing performance and hydrodynamic exposure.

1. Introduction

In the field of pharmaceutical production, bioreactors have proved to be instrumental due to their cost-effectiveness and ability to produce high yields^[1–3], enabling the controlled cultivation of living cells to produce life-saving therapeutics including monoclonal antibodies (mAbs), vaccines, and recombinant proteins at commercially viable scales.^[4] With stirred-tank bioreactors constituting most operated vessels in biopharmaceutical manufacturing, impeller types and configurations have been established to strongly impact mass transfer efficiency and fluid mixing homogeneity, making them essential design considerations for microbial fermentation and mammalian cell-culture processes.^[5] Mammalian cells, such as Chinese hamster ovary (CHO) cells, are particularly sensitive to deviations in dissolved oxygen, pH,

temperature, and nutrient concentration.^[1] Optimizing impeller design to maintain these conditions, while achieving adequate mixing, therefore represents a foundational challenge in bioreactor engineering.

With the growing demand for biomanufacturing capacity, manufacturers have increasingly invested in large industrial-scale bioreactors, with working volumes reaching 5,000–25,000 L, to meet these demands.^[6] At this larger scale, establishing proper parameters becomes more technically complex because geometric and hydrodynamic relationships do not change linearly with vessel size.^[7] Consequently, mixing time, oxygen-transfer capacity, and local hydrodynamic conditions can differ substantially between scales and may contribute to spatial gradients experienced by

suspended cells.^[6,8,9] Mixing time, commonly defined as the time required to reach 95% fluid homogeneity, is therefore a useful hydrodynamic metric to quantify mixing performance.^[10] However, mixing time alone does not establish the magnitude of nutrient or dissolved-oxygen gradients, which additionally depends on cellular uptake, gas-liquid mass transfer, and process operating conditions not represented in a single-phase mixing model.^[11]

Minimizing mixing time is highly desirable for bioreactor operation, as efficient mixing improves overall productivity by ensuring improved mass transfer and reducing heterogeneities experienced by suspended cells. According to fluid dynamics theory, mixing time and impeller speed are shown by the relation (Equation 1):

$$N \cdot \theta_m = K \quad (\text{Eq. 1})$$

where N denotes impeller speed, θ_m denotes mixing time, and K represents a constant dependence on vessel size, geometry, and flow type. This relationship indicates that reducing mixing time requires higher agitation intensity via faster impeller speeds. Current research, such as the work conducted by Kreitmayer *et al.*, provides empirical evidence to substantiate this relationship by drawing a similar correlation between experimentally collected values for mixing time and calculated hydrodynamic stresses.^[12] However, higher impeller speeds consequently result in increased EDR and hydrodynamic shear stresses.^[13,14] The biological consequences of these shear stresses are substantial: increased stress can compromise cell membrane integrity, reduce viability, and diminish product yield and quality.^[15–17] This is especially the case for shear-sensitive mammalian cell lines such as CHO cells. Therefore, studying energy dissipation rate (EDR) and shear stresses in stirred-vessel bioreactors is crucial for understanding the hydrodynamics of the tank and thus, optimizing the vessel for quality product production. Although this tradeoff between mixing performance and viable conditions for cell survival is well documented, few studies have extensively investigated the effects of impeller configurations on mixing and shear, in the context of large-scale industrial vessels.^[18,19] Nadal-Rey *et al.* and Vrábek *et al.* examined the effects of various impeller configurations on bioreactor process performance at multiple industrial scales. However, shear and EDR were not characterized, and only a limited number of configurations were experimentally investigated in each study.^[18,19] As a result, neither study explored whether

alternative impeller arrangements could achieve better mixing performance without proportionally increasing hydrodynamic stress, nor were different impeller combinations and their process performances systemically explored across the scales present in each study.

To build on this research gap, computational fluid dynamics (CFD) will be used as a hydrodynamics simulation and analysis tool. CFD is widely recognized as a valuable tool for characterizing bioreactor properties. In this study, CFD simulations provide detailed visualization of fluid flow patterns within a bioreactor, allowing quantitative assessment of parameters such as shear stress distribution, EDR, and flow velocity.^[20,21] It should be noted that CFD results are subject to uncertainty introduced by turbulence model selection, mesh resolution, and simplifying assumptions.^[22] Importantly, analyzing high working-volume reactors raises complications due to the difficulty, time, and financial costs associated with running physical experimental procedures.^[23] As a standard tool that yields detail and accuracy, utilizing CFD also circumvents the time-related and financial expenses that come with experimental data collection procedures on large-scale vessels.^[24,25]

This study employs COMSOL Multiphysics (v6.2) simulations to systematically evaluate process performance of an industrial-scale, stirred vessel bioreactor, to provide quantitative design guidance for impeller selection. Mixing time, shear rate, shear stress, and EDR are analyzed for varying combinations of Smith impellers and pitched-blade impellers in a nominal capacity of 30,000 L stirred-vessel bioreactor operated at a 20,000 L working volume housing CHO cells. A 3,000 L small-scale vessel, with 2,000 L working volume, using the all-pitched-blade configuration is additionally compared with the large-scale vessel to assess whether the selected condition reproduces the hydrodynamic quantities evaluated here. The simulations use single-phase Newtonian water and do not include cells, gas sparging, oxygen transfer, or culture-dependent rheology. Biological implications are therefore discussed as hypotheses or design considerations rather than direct model outputs.

2. Theory

This study employs the turbulent flow k - ϵ model to simulate bioreactor fluid dynamics. The k - ϵ model was selected for its well-established performance in turbulent stirred-tank flows.^[26] Overarching fluid momentum and continuity are established through the Navier-Stokes

equations provided in Equations 2 and 3:

$$\rho \left(\frac{\partial \mathbf{u}}{\partial t} + (\mathbf{u} \cdot \nabla) \mathbf{u} \right) = -\nabla p + \mu \nabla^2 \mathbf{u} + \rho \mathbf{f} \quad (\text{Eq. 2})$$

$$\nabla \cdot \mathbf{u} = 0 \quad (\text{Eq. 3})$$

where ρ is the fluid density, $\mathbf{u}$ denotes the vector of velocity, t represents time, p is fluid pressure, μ represents dynamic viscosity, and $\mathbf{f}$ denotes the body force acting on the fluid volume.

Equations 4–9 are the governing equations used to compute phenomena relating to and affected by turbulence within the k - ε model:

$$\rho(\mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot (-p\mathbf{I} + \mathbf{K}) + \mathbf{F} \quad (\text{Eq. 4})$$

$$\mathbf{K} = (\mu + \mu_T)(\nabla \mathbf{u} + (\nabla \mathbf{u})^T) \quad (\text{Eq. 5})$$

$$\rho \left(\frac{\partial k}{\partial t} + (\mathbf{u} \cdot \nabla) k \right) = \nabla \cdot \left(\left(\mu + \frac{\mu_T}{\sigma_k} \right) \nabla k \right) + P_k - \rho \varepsilon \quad (\text{Eq. 6})$$

$$\rho \left(\frac{\partial \varepsilon}{\partial t} + (\mathbf{u} \cdot \nabla) \varepsilon \right) = \nabla \cdot \left(\left(\mu + \frac{\mu_T}{\sigma_\varepsilon} \right) \nabla \varepsilon \right) + C_{\varepsilon_1} \frac{\varepsilon}{k} P_k - C_{\varepsilon_2} \rho \frac{\varepsilon^2}{k} \quad (\text{Eq. 7})$$

$$\mu_T = \rho C_\mu \left(\frac{k^2}{\varepsilon} \right) \quad (\text{Eq. 8})$$

$$P_k = \mu_T [\nabla \mathbf{u} : (\nabla \mathbf{u} + (\nabla \mathbf{u})^T)] \quad (\text{Eq. 9})$$

where $\mathbf{I}$ is the identity tensor, $\mathbf{F}$ is the body force vector (the equivalent of $\rho \mathbf{f}$, as denoted in Equation 2), $\mathbf{K}$ is the viscous stress tensor, μ_T is the turbulent viscosity, $(\nabla \mathbf{u})^T$ represents the transpose of the velocity gradient tensor, k is the turbulent kinetic energy, P_k is the turbulence production term, ε is the turbulence dissipation rate, and C_{ε_1} , C_{ε_2} , C_μ , σ_k , and σ_ε are empirical constants. Note that Equation 4 represents the time-averaged form of Equation 2 under turbulent conditions.

Shear rate and shear stress were calculated using Equations 10 and 11:

$$\dot{\gamma} = \sqrt{2 \left[\left(\frac{\partial u}{\partial x} \right)^2 + \left(\frac{\partial v}{\partial y} \right)^2 + \left(\frac{\partial w}{\partial z} \right)^2 + \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right)^2 + \left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right)^2 + \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right)^2 \right]} \quad (\text{Eq. 10})$$

$$\tau = \mu \dot{\gamma} \quad (\text{Eq. 11})$$

where $\dot{\gamma}$ is the shear rate, τ is the shear stress, and u , v , and w are velocity components in the x , y , and z directions respectively. Equation 11 reflects the Newtonian fluid assumption applied in this study, whereby shear stress is directly proportional to shear rate through the dynamic viscosity.

3. Methods

3.1 Bioreactor Geometry

All large-scale simulations were conducted using the bioreactor geometry shown in **Figure 1**, with

dimensions provided in **Table 1**. The vessel has a nominal capacity of 30,000 L and was modeled in COMSOL Multiphysics at a 20,000 L liquid working volume. The vessel has a total height of 8.2 meters (m) and a diameter of 3.0 m (**Figure 1A**). The modeled interior of the vessel (**Figure 1B**) was constructed to include the full complement of internal hardware relevant to mixing performance. It contains a central vertical impeller shaft, four equally spaced, wall-mounted baffles, and two distinct impeller types mounted along the shaft.

3.2 Impeller Configurations

The impeller types used in this study, and their dimensions, are provided in **Figure 2** and **Table 2**, respectively. Two impeller geometries were considered: a pitch-blade impeller with four blades set at a constant pitch angle of 45° (**Figure 2A**), and a Smith turbine with six curved (concave) blades mounted on a central disk (**Figure 2B**). The pitched-blade impeller had a propeller diameter of 1.10 m and a blade width of 0.45 m while the Smith impeller had a disk diameter of 0.83 m, a blade length of 0.28 m, a blade width of 0.10 m, and a blade curvature radius of 0.06 m. Both impeller types were mounted on hubs measuring 0.17 m in diameter and 0.20 m in height.

Each configuration consisted of three impellers positioned at fixed vertical elevations along the central shaft: 5.16 m, 3.14 m, and 1.12 m from the vessel base (**Table 2**). Each of these three positions could be independently occupied by either a Smith turbine or a pitched-blade impeller, resulting in eight possible impeller-type combinations. As shown in **Table 3**, each combination was simulated in this study, allowing the systematic evaluation of impeller type and axial placement, and their effects on mixing performance.

3.3 Mixing Time Model

To compute mixing time, a spherical tracer pulse, with a radius of 0.1 m and a concentration of 1 mol/m³, was introduced below the fluid surface at the coordinate (0.43, -0.37, 7.11) within the modeled fluid domain. The tracer was transported by convection and molecular diffusion using the velocity field obtained from the rotating-frame flow solution. Simulations were performed at an impeller rotational speed of 100 rpm as an intermediary baseline operating condition. With the selected speed, increased agitation and thus, excessive hydrodynamic stresses, could be avoided while still allowing proper mixing. Simultaneously, maintaining

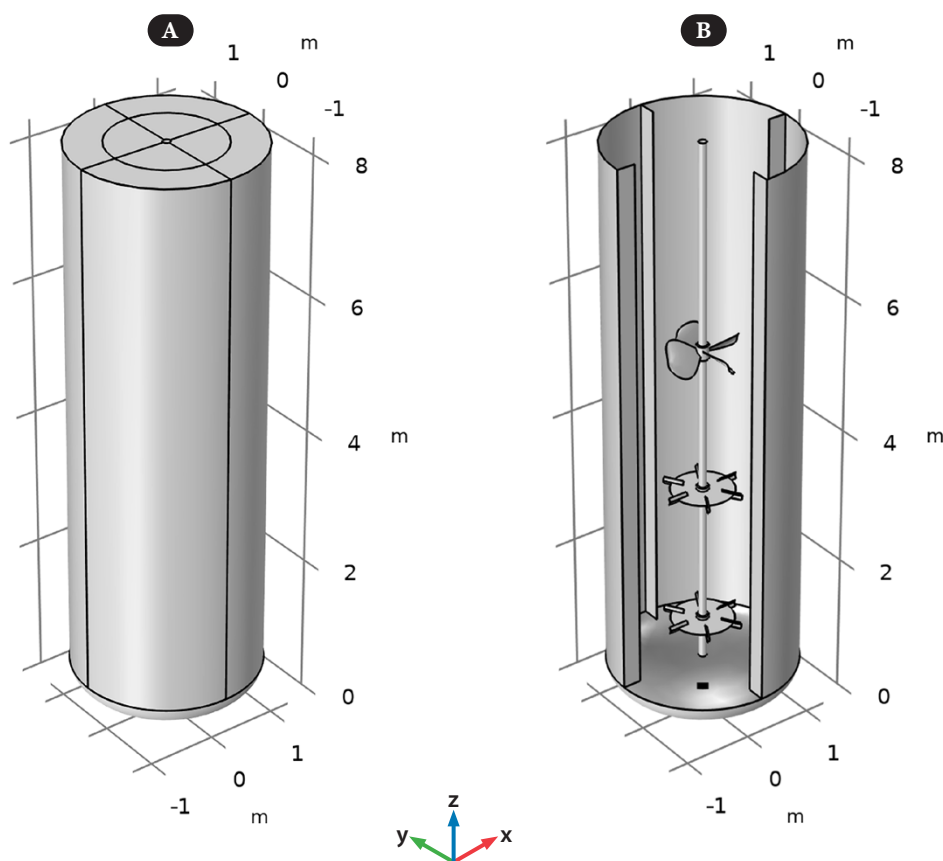


FIGURE 1. Stirred vessel geometries generated in COMSOL Multiphysics.

(A) The exterior view of the entire vessel; and
(B) an interior view including the baffles, impeller shaft, and impellers.

TABLE 1. Stirred vessel geometry parameters.

Parameter	Value	Unit
Tank height	8.2	m
Tank diameter	3.0	m
Number of baffles	4	each
Baffle width	0.3	m
Impeller shaft diameter	0.1	m
Impeller shaft height	8.0	m

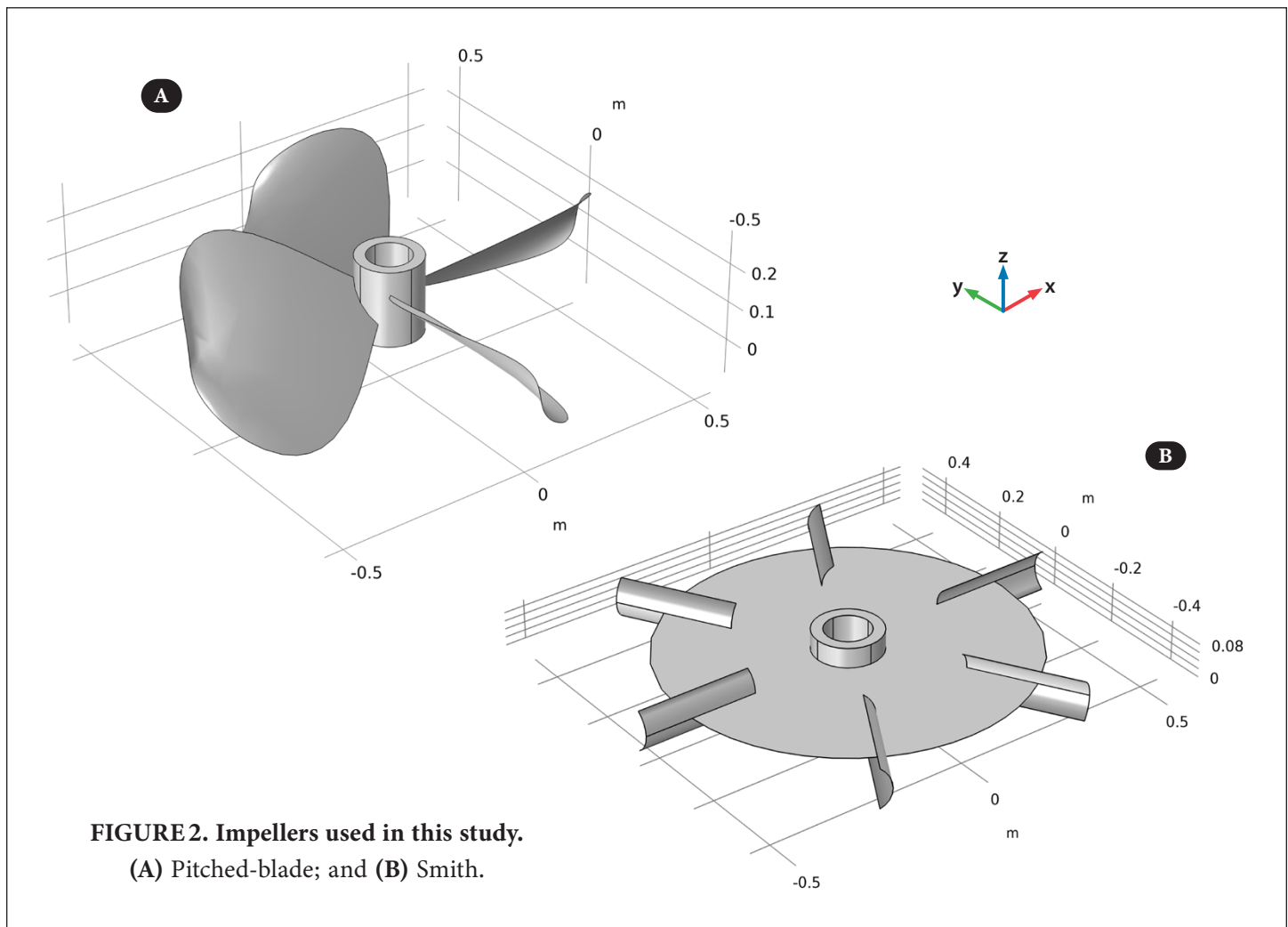


FIGURE 2. Impellers used in this study.
(A) Pitched-blade; and (B) Smith.

TABLE 2. Impeller geometry parameters.

Parameter	Value	Unit
Diameter of propeller hubs	0.17	m
Height of propeller hubs	0.20	m
Pitch angle of pitched-blade propeller blade	45	°
Number of pitched-blade impeller blades	4	each
Width of pitched-blade impeller blades	0.45	m
Pitched-blade propeller diameter	1.1	m
Length of Smith impeller blades	0.28	m
Number of Smith impeller blades	6	each
Smith impeller blade curvature radius	0.06	m
Width of Smith impeller blade	0.10	m
Smith impeller disk diameter	0.83	m
Vertical elevation of first (top) impeller	5.16	m
Vertical elevation of second (middle) impeller	3.14	m
Vertical elevation of third (bottom) impeller	1.12	m

TABLE 3. Simulated impeller combinations in vessels.

Impeller Combination/Arrangement				
		Top	Middle	Bottom
Configuration Number	1	Pitched-blade	Smith	Smith
	2	Pitched-blade	Pitched-blade	Smith
	3	Pitched-blade	Smith	Pitched-blade
	4	Smith	Pitched-blade	Pitched-blade
	5	Smith	Smith	Pitched-blade
	6	Smith	Pitched-blade	Smith
	7	Pitched-blade	Pitched-blade	Pitched-blade
	8	Smith	Smith	Smith

this speed across all simulations allows for systematic analysis of results that can be attributed to the effects of specific impeller arrangements rather than operating speeds. The time-dependent transport solver used adaptive time-stepping with a relative tolerance of 0.005. COMSOL automatically adjusted the internal solver time step to maintain specified error tolerances. This approach enables smaller time steps during periods of rapid concentration change and larger time steps as the field became more homogenous, while solution outputs were recorded at fixed 1-second (s) intervals.

To determine when the tracer concentration was dispersed at 95% homogeneity, the formula for coefficient of variation (*CoV*) was utilized as given below:

$$CoV = \frac{\sigma}{\bar{c}} \quad (\text{Eq. 12})$$

where σ represents the standard deviation of concentration and $\bar{c}$ denotes the mean concentration throughout the reactor. As a dimensionless measure of concentration non-uniformity, lower *CoV* values indicate higher levels of homogeneity and vice versa. As is commonly employed in industrial practice, this study considered a reactor to reach 95% homogeneity once the *CoV* decreased to 0.05 and below.^[27,28]

3.4 Meshing Strategy

To assess mesh sensitivity of the mixing-time endpoint, three mesh designs were evaluated for each impeller configuration, as summarized in **Table 4**. In the baseline mesh (Mesh Design 2), a mesh quality of *normal*

was applied to the tank walls and global domain, *fine* was applied to the rotating domain and baffles, and *extremely fine* was applied to all impellers. The impellers, rotating domain, and baffles have the greatest impact on fluid flow and thus, were more finely meshed than the remainder of the bioreactor, requiring higher spatial resolution to accurately resolve velocity gradients and mixing behavior near the impeller blades.

In addition, to verify that the collected mixing time data were independent of the mesh resolution, each of the eight impeller configurations was additionally simulated using two alternative mesh designs (Mesh Designs 1 and 3), which respectively coarsened and refined the mesh quality across all tank geometry regions relative to the baseline (**Table 4**). The resulting number of mesh elements for each configuration under each mesh design is reported in **Table 5**. A comparison of mixing time results across all three mesh designs was used to confirm mesh independence of the reported results.

The standard *k-ε* turbulence model was employed to represent turbulent flow throughout the reactor. Water at 20°C was modeled as an incompressible Newtonian fluid with constant density and viscosity. No-slip boundary conditions were applied to all reactor walls, baffles, and impeller surfaces. The impeller region was modeled as a rotating domain with a rotational speed of 100 rpm, while the remaining fluid domain remained stationary. A tracer was introduced into the reactor using an initial concentration distribution, and transport was governed by convection and molecular diffusion with a diffusion

TABLE 4. Varying mesh quality levels for geometry and domains of the vessel.

		Mesh quality at corresponding mesh level		
		Mesh Design 1	Mesh Design 2	Mesh Design 3
Tank Geometry	Global	Normal	Normal	Fine
	Rotating Domain	Fine	Fine	Extra Fine
	Impeller	Fine	Extremely Fine	Extremely Fine
	Baffles	Normal	Fine	Fine
	Tank Walls	Normal	Normal	Fine

TABLE 5. Number of mesh elements for each configuration according to the applied mesh design.

		Mesh elements × 10 ⁶ at corresponding mesh level		
		Mesh Design 1	Mesh Design 2	Mesh Design 3
Configuration Number	1	0.403224	1.906416	3.644485
	2	0.402424	1.701167	3.415521
	3	0.403229	1.695204	3.412949
	4	0.402466	1.697504	3.413962
	5	0.401853	1.904099	3.642363
	6	0.402114	1.908679	3.647339
	7	0.397210	1.498776	3.149226
	8	0.402012	2.116328	3.871775

TABLE 6. Small-scale vessel geometry parameters.

Parameter	Value	Unit
Tank height	3.8	m
Tank diameter	1.2	m
Number of baffles	4	each
Baffle width	0.1	m
Impeller shaft diameter	0.05	m
Impeller shaft height	3.6	m
Diameter of propeller hubs	0.08	m
Height of propeller hubs	0.09	m
Pitch angle of pitched-blade propeller blade	45	°
Number of pitched-blade impeller blades	4	each
Width of pitched-blade impeller blades	0.21	m
Pitched-blade propeller diameter	0.5	m
Vertical elevation of first impeller	2.14	m
Vertical elevation of second impeller	1.20	m
Vertical elevation of third impeller	0.26	m

coefficient of $1 \times 10^{-9} \text{ m}^2/\text{s}$. No-flux boundary conditions were applied to all reactor boundaries.

The flow field was first established using a *frozen rotor* solution to obtain a converged velocity in the rotating frame of reference. That velocity field was subsequently used for the time-dependent *transport of diluted species* calculation which was used to estimate mixing time. The governing equations were solved using a segregated iterative solver with the COMSOL default GCRO-DR linear solver. *Frozen rotor* was selected because it provides a computationally tractable, internally consistent flow field for comparing the eight impeller arrangements in a vessel of this size. However, because the rotor-stator relative position is fixed, this approach does not resolve blade-passing transients or time-dependent rotor-stator interactions. Mixing-time values should therefore be interpreted as predictions based on a steady rotating-frame velocity field rather than a fully transient, sliding-mesh representation.

3.5 Small-Scale Simulation

A 3,000 L small-scale vessel with a working volume of 2,000 L was evaluated using Configuration 7 (three pitched-blade impellers) and compared with the large-scale 30,000 vessel. The scale comparison was designed to preserve the selected impeller arrangement and power

input per unit volume (P/V). Hydrodynamic outputs compared between scales were mixing time, volume-average and maximum shear stress, and volume-average and maximum EDR. As outlined in **Table 6**, all geometry components and aspect ratios were kept constant, with the only applied change being a scale ratio of 0.464 to achieve a nominal volume of 3,000 L.

4. Results and Discussion

4.1 Mesh Independence Study

Mixing time was evaluated for all eight impeller configurations at three mesh resolutions to assess both the comparative mixing performance of each design and the mesh independence of the reported values.

As shown in **Figure 3A**, mixing times (using the Mesh Design 2 baseline) ranged from 214 s for Configuration 7 (all pitched-blade) to 316 s for Configuration 8 (all Smith), representing roughly a 48% difference between the fastest and slowest mixing configurations. Configurations with more pitched-blade impellers generally exhibited shorter mixing times. Configuration 7 mixed the fastest, followed by Configurations 2–4 (each containing two pitched-blade impellers, ranging from 225–241 s), while Configurations 5 and 6 (each containing a single pitched-blade, ranging from 262–264 s) and Configuration 8 (no pitched-blade) required progressively longer times

to reach 95% homogeneity. This trend is consistent with the strongest axial circulation generated by pitched-blade impellers relative to the more radially dominant discharge of the Smith turbine, promoting faster top-to-bottom bulk fluid exchange throughout the vessel.

Mesh independence was verified by repeating each configuration simulation at a coarser (Mesh Design 1) and finer (Mesh Design 3) mesh resolution relative to the baseline (Figure 3B). Mixing times increased slightly with mesh refinement across all eight configurations, with Mesh Design 3 producing mixing times 2.1–4.9% higher than the Mesh Design 2 baseline, and Mesh Design 1 producing mixing times 1.2–3.9% lower. All

deviations fell within, or very close to, a $\pm 5\%$ reference band around the baseline, with the largest deviation (4.9%, Configuration 6) still inside this range. Critically, the relative ranking of mixing times across the eight configurations was preserved at every mesh resolution tested, indicating that while absolute mixing time values shift modestly with mesh density, the comparative conclusions drawn between impeller configurations are robust to mesh resolution. On this basis, Mesh Design 2 was adopted as the baseline mesh for all subsequent analyses in this study.

4.2 Flow Field Analysis

Velocity magnitude contours, overlaid with in-plane velocity vectors on a vertical slice through the vessel centerline, were generated for each of the eight impeller configurations to characterize the resulting flow fields (Figures 4A–H, corresponding to Configurations 1–8 in Table 3). Across all configurations, the highest local velocity magnitudes (approximately 6–8 m/s) were consistently found at the closed region of the impeller blades. Velocity decreased sharply, with radial and axial distance from each impeller toward the bulk fluid and the tank walls.

The two impeller types produced visually and structurally distinct local flow patterns at each elevation. Positions occupied by a pitched-blade impeller generated tight, looped recirculation zones immediately above and below the blade plane, which is characteristic of the strong axial component imparted by the pitched geometry (e.g., the topmost impeller in Figure 4A, Configuration 1: pitched/Smith/Smith). Positions occupied by a Smith turbine instead produced broader, radially dominant discharge patterns, with fluid ejected outward toward the tank wall and forming more diffused counter-rotating vortices above and below the disk (e.g., the lower two impeller planes in Figure 4A).

Where two pitched-blade impellers were positioned at adjacent elevations, their individual loop structures merged into a single, elongated axial circulation cell expanding to both impeller planes, which produced stronger top-to-bottom bulk fluid exchange over that section of the

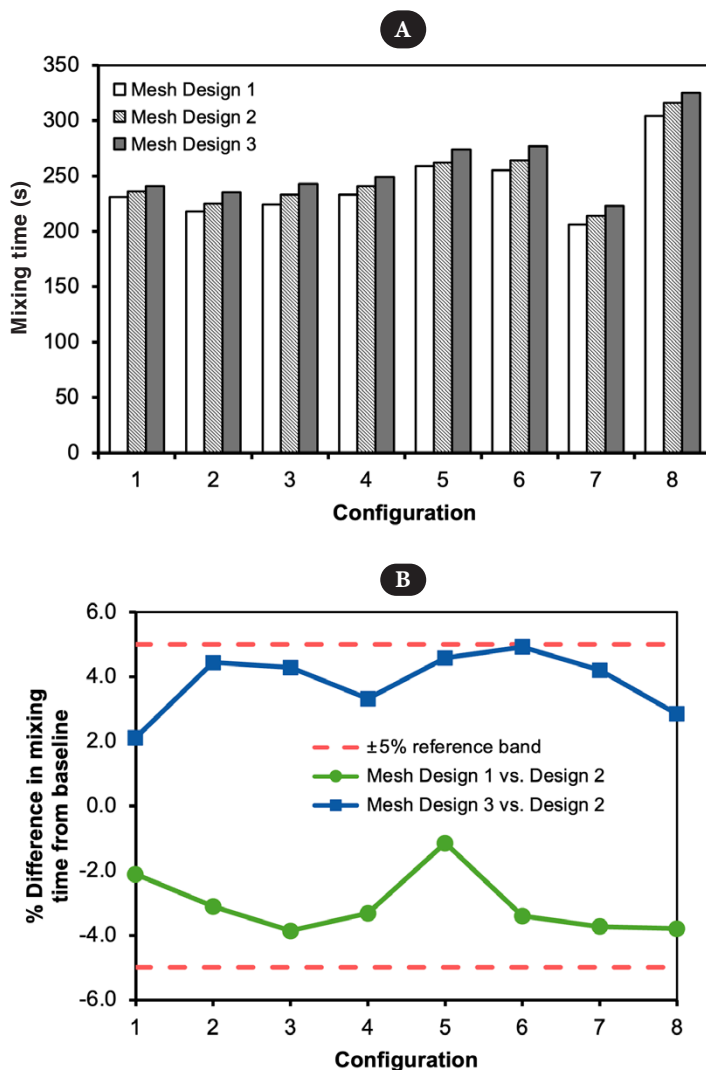


FIGURE 3. Mesh-independence assessment based on mixing time.

(A) Mixing time for the eight impeller configurations using three mesh designs; and (B) percentage difference in mixing time relative to Mesh Design 2.

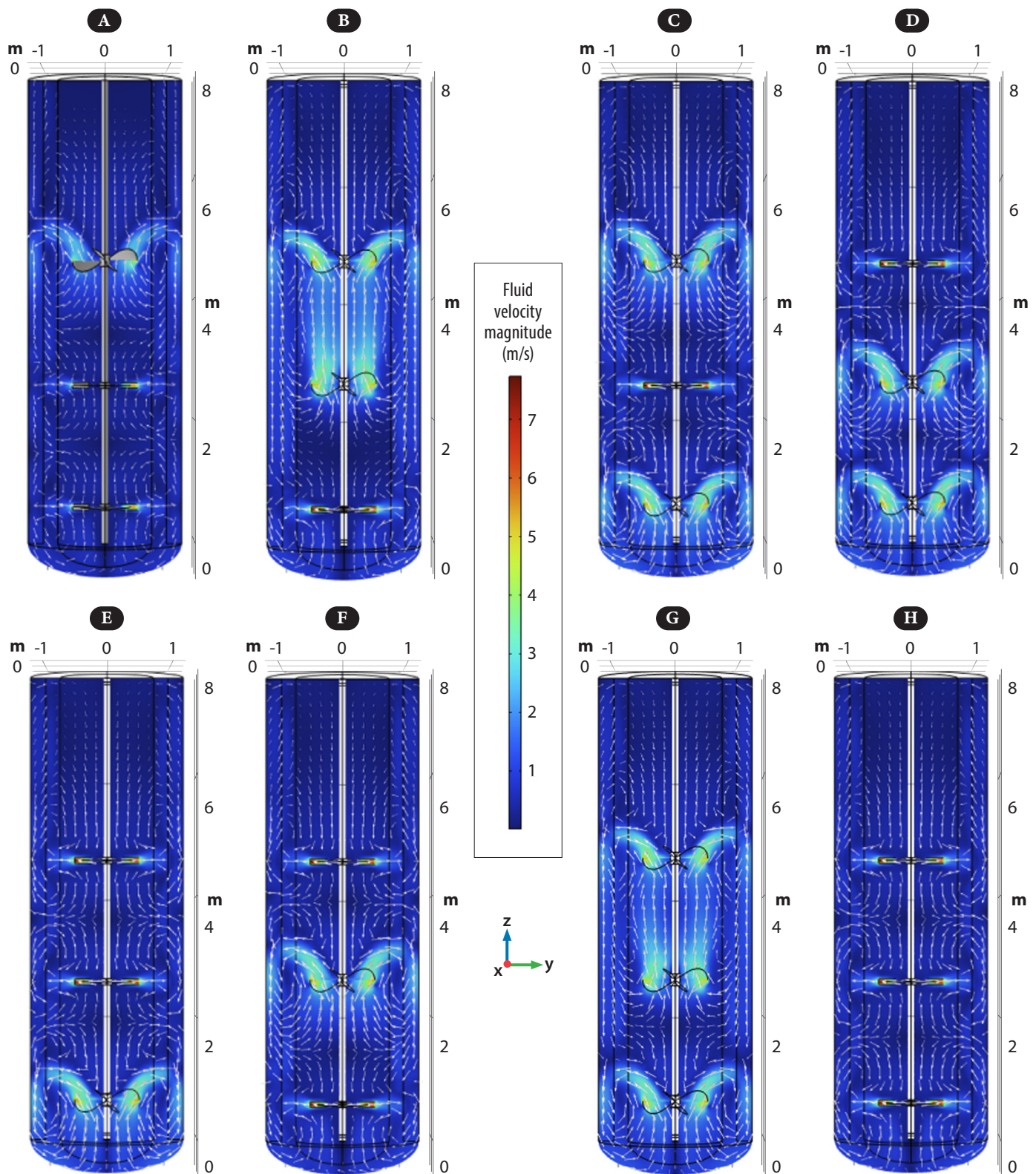


FIGURE 4. Velocity magnitude contours (m/s) with overlaid in-plane velocity vectors on a vertical slice through the vessel centerline.

Illustrations (A–H) correspond to configurations 1–8, respectively, as listed in Table 3.

vessel (**Figures 4B–D and F**, Configurations 2–4 and 6). This effect was most elevated in the all-pitched-blade configuration (**Figure 4G**, Configuration 7) where the three impeller planes combined into a continuous axial circulation pattern extending nearly the full height of the vessel, with the largest observed region of elevated velocity magnitude among all the configurations tested. In contrast, the all-Smith configuration (**Figure 4H**, Configuration 8) exhibited three largely independent radial flow zones with comparatively weak interaction between impeller planes and lower peak velocities in the bulk region between impellers.

In mixed configurations where pitched-blade and Smith impellers alternated along the shaft (**Figures 4C, E, and F**, Configurations 3, 5, and 6), each impeller generated a flow pattern typical to its own type, largely independent of its neighbors. Axial recirculation loops formed at pitched-blade elevations, while radial discharge patterns formed at Smith impeller elevations, with little mixing of the two flow types between adjacent planes.

These circulation patterns have direct implications for cell suspension and distribution within the bioreactor. The strong, continuous top-to-bottom axial circulation generated by adjacent or fully pitched-blade arrangements (**Figures 4B–D, F, and G**) is favorable for maintaining cells in homogeneous suspension throughout the vessel height, reducing the likelihood of settling, sedimentation, or the formation of localized concentration gradients in nutrients, dissolved oxygen, or metabolic byproducts. In contrast, the all-Smith configurations (**Figure 4H**) show weaker fluid exchange between impeller zones, with flow largely confined to separate circulation loops at each elevation. This compartmentalization means fluids at one impeller level mixes relatively little with fluid at the levels above or below it, which could allow different environments to develop at each height and create stagnant zones between impeller planes, especially near the vessel base. Mixed configurations (**Figures 4C, E, and F**) fall between these two extremes. In these settings, vertical exchange is strong near each pitched-blade impeller but weaker near each Smith impeller. This indicates that not only the type of impeller used, but also where each type is placed within the vessel, affects how uniformly cells and nutrients are distributed throughout the culture volume.

4.3 Shear Stress Analysis

The spatial distribution of shear stress was examined for each impeller configuration (**Figure 5**). In all cases,

the maximum shear stresses were localized near the impeller blades and discharge regions while most of the vessel remained at substantially lower stress levels.

These spatial differences were quantified using volume-average and maximum shear stress and were evaluated as functions of impeller composition to characterize the bulk and localized hydrodynamic shear environments generated by the eight configurations (**Figure 6**). Volume-average shear stress increased with the number of pitched-blade impellers. Configurations containing a greater proportion of axial-flow impellers produce higher bulk shear stress throughout the vessel. Configuration 7 (three pitched-blade impellers) and Configuration 3 (two pitched-blade impellers) exhibited the highest volume-average shear stress, while Configuration 8 (three Smith impellers) and Configuration 1 (one pitched-blade and two Smith impellers) exhibited the lowest (**Figure 6A**). This trend is consistent with the more extensive axial circulation generated by pitched-blade impellers relative to the radially confined discharge of the Smith turbine (**Figure 4**). However, Configuration 1 was a notable exception to this trend. Despite containing a pitched-blade impeller, it exhibited the lowest volume-average shear stress of all eight configurations, indicating that the axial position and arrangement of impeller types, rather than impeller type alone, influences bulk shear stress.

In contrast to volume-average shear stress, maximum shear stress showed a weak negative correlation with volume-average shear stress across configurations (**Figure 6B**). Configuration 1 exhibited the highest maximum shear stress of any configuration (1.51 pascals [Pa]), approximately double that of the next-highest configuration, despite having the lowest volume-average value. Conversely, Configuration 7 exhibited the lowest maximum shear stress (0.61 Pa) despite having the highest volume-average shear stress. This divergence indicates that Configuration 1 contains a localized shear spot rather than a broadly elevated shear field. This is consistent with the isolated, unmerged circulation loop surrounding its single pitched-blade impeller (**Figure 4A**). The all-pitched-blade configuration (Configuration 7), by comparison, has its three merged circulation loops distribute energy dissipation more evenly throughout the vessel rather than concentrating it at a single location.

The maximum shear stresses predicted for Mesh Design 2 ranged from 0.51–1.51 Pa. These values are substantially below the maximum tolerable

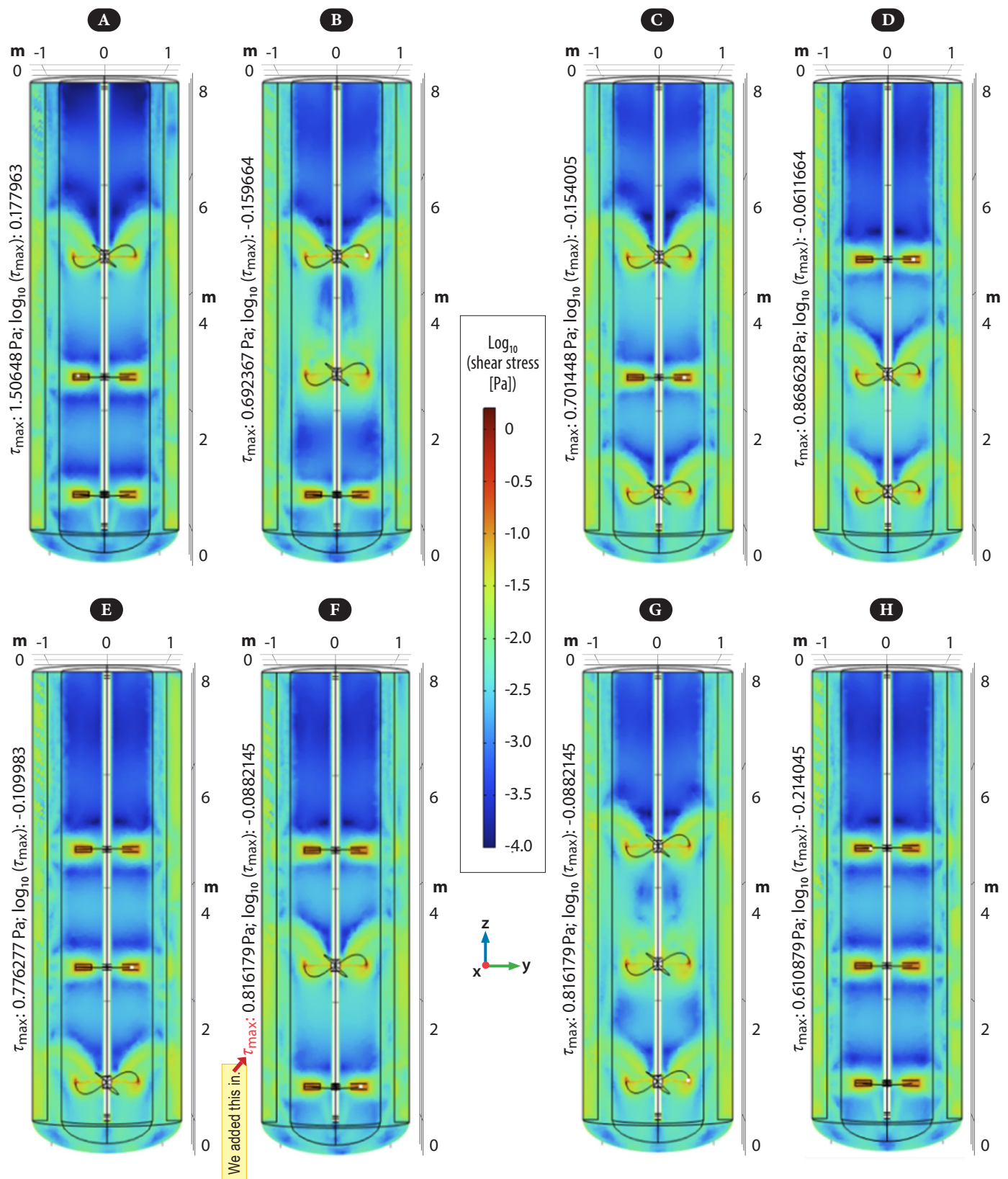


FIGURE 5. Spatial distribution of shear stress for the eight configurations using Mesh Design 2.

Contour illustrations (A-H) show $\log_{10}$ shear stress in Pa. Maximum shear stress for each configuration is reported adjacent to its corresponding location and highlighted by a white square marker.

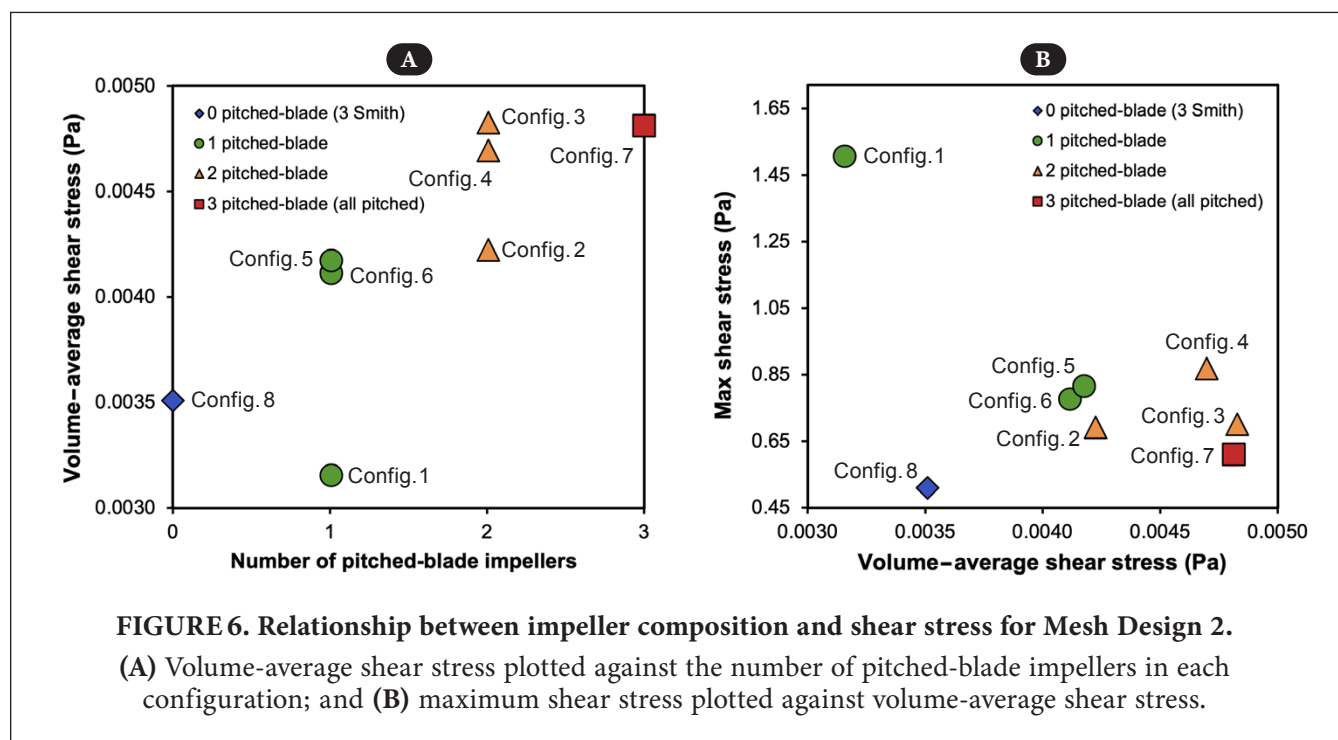


FIGURE 6. Relationship between impeller composition and shear stress for Mesh Design 2.

(A) Volume-average shear stress plotted against the number of pitched-blade impellers in each configuration; and (B) maximum shear stress plotted against volume-average shear stress.

hydrodynamic stress of 32.4 ± 4.4 Pa reported for the CHO cell evaluated by Neunstoecklin *et al.*, corresponding to approximately 1.6–4.7% of that reported range.^[29] This comparison suggests a low risk of acute hydrodynamic damage under the simulated operating conditions. Godoy-Silva *et al.* similarly found that GS-CHO cells were relatively resistant to repeated hydrodynamic exposure.^[30] They identified a shear stress threshold of approximately 6 Pa, where a measurable change in recombinant protein glycosylation profile occurs in GS-CHO cells. At a substantially higher threshold of approximately 150 Pa, a fatal cellular response is triggered. On this basis, none of the configurations tested are expected to produce shear stress levels associated with reduced viability or altered product quality under the operating conditions simulated.

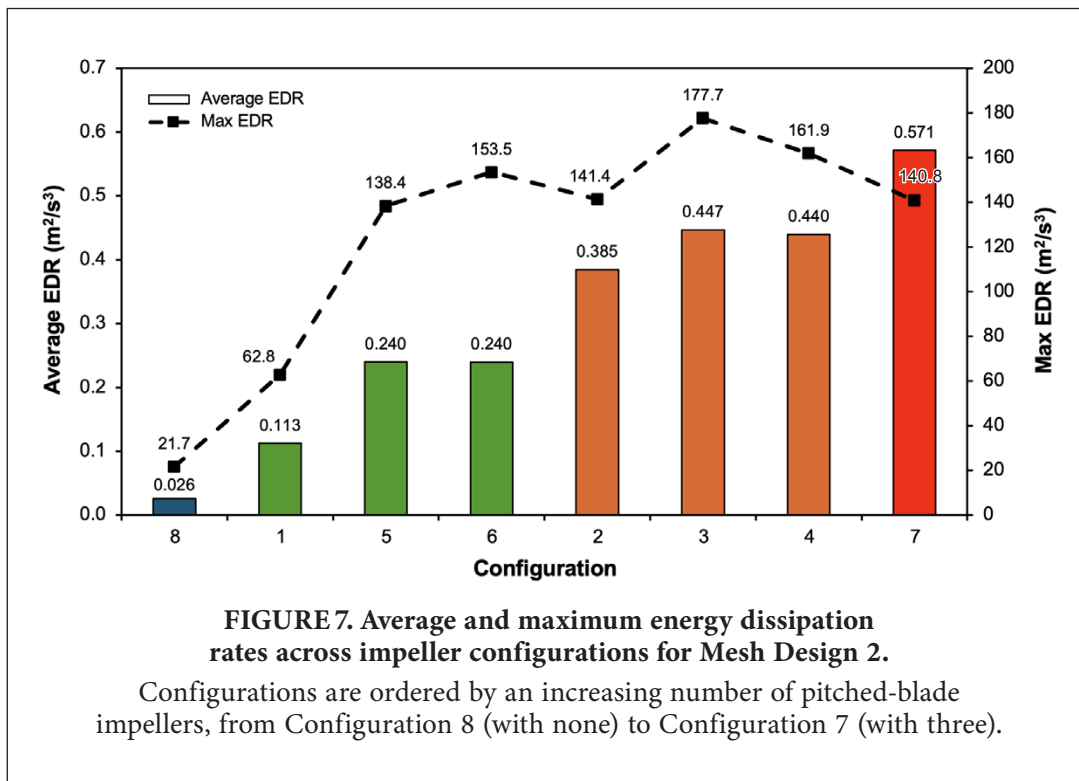
Average and maximum EDR were evaluated across the eight impeller configurations to characterize both the overall intensity of turbulent dissipation and the magnitude of localized dissipation peak (Figure 7). Volume-average EDR increased strongly with the number of pitched-blade impellers. Configuration 8, containing three Smith turbines and no pitched-blade impellers, produced the lowest average EDR at $0.026 \text{ m}^2/\text{s}^3$, whereas Configuration 7, containing three pitched-blade impellers, produced the highest value at $0.571 \text{ m}^2/\text{s}^3$. Configurations containing one pitched-blade impeller exhibited intermediate average

EDR values of $0.113\text{--}0.240 \text{ m}^2/\text{s}^3$, while configurations containing two pitched-blade impellers produced higher values of $0.385\text{--}0.447 \text{ m}^2/\text{s}^3$.

Although the overall trend indicates that increasing pitched-blade content raises the vessel-average dissipation rate, the impeller counts alone did not fully account for the differences among configurations. Configurations 1, 5, and 6 each contain one pitched-blade impeller, yet Configuration 1 produced an average EDR approximately half that of Configurations 5 and 6. Similarly, the three configurations containing two pitched-blade impellers differed in average EDR despite having the same overall impeller composition. These differences indicate that impeller sequence, vertical position, and interactions between the circulation region also influence how turbulent energy is distributed throughout the vessel.

Maximum EDR followed a less consistent relationship with pitched-blade count. The correlation was positive but did not reach statistical significance. Configuration 3 produced the highest maximum EDR at $177.7 \text{ m}^2/\text{s}^3$, followed by Configuration 4 at $161.9 \text{ m}^2/\text{s}^3$, and Configuration 6 at $153.5 \text{ m}^2/\text{s}^3$. Configuration 7, despite producing the highest volume-average EDR, had a lower maximum of $140.8 \text{ m}^2/\text{s}^3$. Configuration 8 produced both the lowest average and the lowest maximum EDR, with a maximum value of only $21.7 \text{ m}^2/\text{s}^3$.

Average and maximum EDR were positively correlated



across the configurations, indicating that configurations with a more energetic bulk flow generally also generated larger localized dissipation peaks. However, the different configuration rankings demonstrate that these two metrics capture distinct aspects of the hydrodynamic environment. Average EDR describes the overall level of turbulent dissipation within the vessel, whereas maximum EDR identifies the most intense, localized region. Overall, increasing the number of pitched-blade impellers raised average EDR more consistently than maximum EDR. This suggests that pitched-blade impellers primarily increase the distributed turbulent energy

within the vessel, while localized maximum remains dependent on the specific arrangement and interaction of the impellers.

4.4 Scale Comparison

The hydrodynamic environment of the 3,000 L small-scale bioreactor was compared with that of the 30,000 L vessel using Configuration 7, which contains three pitched-blade impellers at both scales (**Table 7**). Despite the matched impeller configuration, mesh-design approach, and power input to unit volume ratio, the two simulations produced substantially different shear,

TABLE 7. Hydrodynamic comparison of 30,000 L bioreactor (Configuration 7) and 3,000 L small-scale vessel.

		Bioreactor Scale	
		30,000 L	3,000 L
Metric	Volume average shear stress (Pa)	0.005	0.027
	Maximum shear stress (Pa)	0.611	1.079
	Average EDR (m^2/s^3)	0.571	10.899
	Maximum EDR (m^2/s^3)	140.8	450.0
	Mixing time (s)	214	51

EDR and mixing characteristics. The 3,000 L vessel consistently exhibited greater hydrodynamic intensity and a considerably shorter mixing time than the larger system.

Volume-average shear stress increased from 0.005–0.027 Pa, corresponding to a 5.4-fold increase. In contrast, maximum shear stress increased by only 1.77-fold, reaching 1.079 Pa in the 3,000 L vessel. Thus, the small-scale vessel experienced a much larger increase in the volume-average shear environment than in the localized maximum.

The scale effect was even more pronounced for EDR. Average EDR increased from 0.571–10.899 m²/s³, and maximum EDR increased from 140.8–450.0 m²/s³. These results indicate that the 3,000 L condition produces a more energetic bulk flow field, while the localized dissipation maximum increases less strongly. The small-scale vessel therefore does not reproduce the larger vessel simply by preserving the same impeller arrangement. Instead, it subjects the fluid to markedly greater average shear and turbulent dissipation.

The increased hydrodynamic intensity in the 3,000 L vessel was accompanied by faster mixing. Mixing time decreased from 214 s in the 30,000 L vessel to 51 s in the small-scale 3,000 L vessel, representing a 76% reduction. The shorter mixing time is consistent with the much higher average EDR, because greater energy dissipation generally promotes faster bulk circulation and homogenization. This improved mixing performance therefore ends at the cost of increased average shear and turbulent exposure.

Overall, the current 3,000 L condition does not provide direct hydrodynamic equivalence to the larger 30,000 L bioreactor. Consequently, the suitability of this condition as a small-scale model depends on the process attribute selected for matching. Matching mixing time, average EDR, maximum EDR, shear exposure, and other parameters such as oxygen-transfer performance simultaneously may not be possible.

5. Conclusion

This study used CFD to compare eight impeller configurations in a 30,000 L stirred-tank bioreactor. Configurations containing more pitched-blade impellers generally produce stronger axial circulation and shorter mixing times. Configuration with three pitched-blade impellers achieved the fastest mixing, while configuration with three Smith turbines mixed most slowly because its flow remained more confined

to separate radial circulation zones.

Improved mixing was accompanied by higher volume-average shear stress and EDR. However, the maximum values did not follow the same trend. Configuration 1, with one pitched-blade impeller on top and two Smith turbines at the bottom, produced the highest maximum shear stress despite having the lowest volume-average shear stress, showing that low average stress does not exclude the presence of localized spots. Therefore, mixing times, average hydrodynamic conditions, and localized maximums should be evaluated together when selecting an impeller arrangement.

The scale comparison further showed that preserving the same three pitched-blade configuration did not produce hydrodynamic equivalence between the 30,000 L and 3,000 L vessels. The 3,000 L vessel mixed 76% faster but exhibited substantially higher average shear stress and EDR. These findings demonstrate that bioreactors' performance with different scales cannot rely on geometric similarity alone and must be based on a clearly selected process criterion. Overall, pitched-blade impellers improved bulk mixing, whereas Smith turbines reduced average hydrodynamic intensity. The optimal configuration therefore depends on the required balance between rapid homogenization and controlled shear exposure.

To further understand the effects of impellers on the fluid dynamics of large-scale bioreactors, future research could focus on three key areas. First, additional mixing factors not addressed in this study, particularly those related to gas flow could be analyzed. Metrics such as oxygen transfer rate (OTR) and volumetric mass transfer coefficient (k_La) are critical for assessing cell viability based on oxygen availability and could offer deeper insight into how specific impeller designs affect cell viability. Second, the same flow variables examined here could be systematically tested across fluids with different rheological properties (e.g., non-Newtonian or high viscous fluids), providing a more comprehensive understanding of how impellers affect fluid dynamics and mass transfer at industrial scale. Finally, several parameters beyond volumetric power consumption can be held constant during scale-up, including impeller speed, tip speed, mixing time, and Reynolds number. Systematically studying each of these scaling parameters would help characterize how maintaining one constant influences another aspect of a bioreactor's fluid dynamics.

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About the Authors

Emmanuel Yu¹, Xian Wu¹, and Chi-Ming Yu^{1*}

1. Manufacturing Science and Technology, Boston Institute of Biotechnology, LLC,
225 Turnpike Road, Southborough, Massachusetts 01772 USA

***Corresponding Author:**

Chi-Ming Yu, PhD, Senior Director,
[Boston Institute of Biotechnology, LLC](#)
Email: chiming.yu@bostonbib.com