

Visualization Approaches for Stirred Tank Bioreactors

Armands Bušs
Institute of General Chemical
Engineering
Riga Technical University
Riga, Latvia
armands.buss@rtu.lv

Normunds Jēkabsons
Institute of Physics
University of Latvia
Salaspils, Latvia
normunds@jesystems.eu

Artūrs Šuleiko
JSC Biotehniskais centrs
Latvian State Institute of Wood
Chemistry
Riga, Latvia
arturs.suleiko.00@rpg.lv

Dagnija Loča
Institute of General Chemical
Engineering
Riga Technical University
Riga, Latvia
dagnija.loc@rtu.lv

Juris Vanags
JSC Biotehniskais centrs
Latvian State Institute of Wood
Chemistry
Riga, Latvia
juris.vanags@rtu.lv

Abstract— Computational Fluid Dynamics (CFD) is the analysis of fluid behaviour employing numerical solution methods. Using CFD it is possible to analyse simple and complex fluid-gas, fluid-fluid or fluid-solid interactions. Fluid dynamics is described with laws of physics in the form of partial differential equations also known as Navier-Stokes equations. Sophisticated CFD solvers transform these laws into algebraic equations which are solved by numerical methods. In this paper Ansys CFX and Fluent analysis systems as research methods are used to visualize flow patterns in a stirred tank bioreactor. The results obtained are informative and can be used to improve the yield of biomass. CFD analysis can save time and aid fluid system designing process. This approach is cheaper and faster compared to conventional build-and-test process. However, it should be noted that CFD analysis results are as accurate as the level of skill possessed by a CFD engineer therefore there are still place for hands-on testing. Authors have developed a stirred tank model and visualized flow patterns. The research presents experimental computation methods and the model setup key parameters. The developed model allows to predict flow patterns inside stirred systems and evaluate efficiency of the mixing process by eddy frequency, shear strain rate and power input.

Keywords— Ansys CFX/Fluent, CFD, bioreactor, stirred tank.

I. INTRODUCTION

Stirred tank bioreactors are used to produce a variety of products across several industries. Many ingredients for the food industry are made through fermentation [1]. For instance, bioreactors are used in the manufacture of antibiotics (e.g., penicillin, lovastatin). Bioethanol has gained importance as a viable alternative fuel, and it is made from fermented agricultural products and waste (e.g., potato starch, wheat, sugar beet). Cell-culture bioreactors lie at the heart of the processes used to produce large-molecule, protein-based therapeutics. Therefore, the importance of scale-up of bioreactors for meeting higher demands of quantity and efficiency of production becomes paramount. Design, construction, and evaluation of bioreactors for larger scale production can be costly and time-consuming endeavour. Some critical limiting factors are fluid mechanics effects (e.g., non-ideal mixing, nutrient and oxygen distribution, mass transfer). Thus, before building a large-scale system it is wise to investigate and visualize a smaller system. The investigation tool used in this research is Ansys CFX simulation software.

Computational fluid dynamics (CFD) refers to solving transport equations for fluid flow, heat and mass transfer quantities using numerical methods. The approach frames a 3-dimensional model and fills the fluid region with large number of finite volumes that are all connected to each other in a form of finite

Print ISSN 1691-5402

Online ISSN 2256-070X

<http://dx.doi.org/10.17770/etr2019vol3.4077>

© 2018 The Author(s). Published by Rezekne Academy of Technologies.

This is an open access article under the [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

volume (or finite element) mesh. On each one of the mesh elements, momentum, the basic conservation of mass and heat transfer is solved. The underlying equations that govern the solution are called Navier-Stokes equations [2] where the global conservation of the mentioned quantities above is satisfied.

In simple words, CFD models quite complex unit operations as a network of well-mixed compartments that adapt to the boundaries of fluid flow geometry. When set up properly this approach works on any conventional geometry, including sliding and moving parts on a very fundamental level. The number of mixed compartments can be small or large providing that the fine spatial resolution of flow represents related processes occurring within the basic steps in process. The main benefit of this modelling approach is increased process understanding and insight into a unit operation from modelling perspective. CFD technology has been and still is widely used in the automotive and aerospace industries. It entered the chemical industries in the middle of nineties along with development of more accessible simulation software that can be run on a PC. The principle “make it right the first time” is desired outcome for many industries as it offers that competitive edge and saves resources. Therefore, modeling fits in as a virtual laboratory before expensive prototypes are built. In the past two decades, CFD has seen increased interest in the fermentation and bioprocessing area, where insight into fluid flow and related phenomena (e.g., turbulence) can assist in risk management associated with mixing zones in bioreactors.

This research investigates a 5L working volume laboratory stirred vessel (298 K, 1 atm) setup with double custom build magnetically coupled Rushton impellers (Fig. 1) and a cell-culture fluid that resembles physical properties of water.

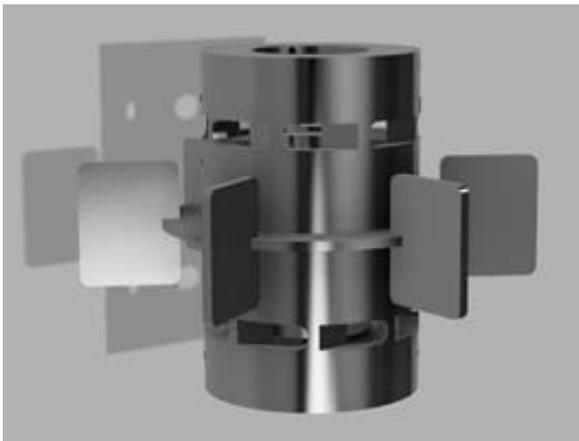


Fig. 1. Magnetically coupled impeller designed and rendered in Autodesk Fusion 360.

In this research we are answering and explaining the following questions:

1. What is the flow pattern and average liquid velocity in bulk liquid?

2. What is average water turbulence eddy frequency in bulk liquid?
3. What is minimum fluid velocity in a laminar region inside a thin slit between the shaft and rotor?
4. What is the smallest turbulence length scale (eddy size) in bulk liquid?
5. What is the shear strain rate in the liquid?

To answer these questions, we use numerical modelling approach as experimental setup is hardly constructible and due to lack of specialised equipment not possible.

The key setup values are arranged in TABLE I.

TABLE I.

Stirred tank geometry parameters		
Baffle width	0.160	[m]
Impeller blade height	0.020	[m]
Impeller blade width	0.016	[m]
Impeller blades	6	[-]
Impeller diameter	0.078	[m]
Number of baffles	3	[-]
Shaft diameter	0.019	[m]
Shaft length	0.450	[m]
Tank diameter	0.155	[m]
Tank height	0.350	[m]

II. MATERIALS AND METHODS

Ansys CFX a high-performance computational fluid dynamics (CFD) software tool is used to visualize and answer the questions above. As well as Fluent, CFX is recognized for its outstanding accuracy, robustness and speed when simulating turbomachinery (e.g., pumps, fans, turbines and stirred tanks). In the core of CFX is a cell vertex finite volume code similar to Fluent but with more modern look and feel GUI.

The geometry and mesh of the reactor was drawn in SpaceClaim 3D modelling software and Ansys mesh generator. The mesh consists of tetragonal elements in the liquid region and hex-dominant elements in the air region. The near wall inflation was set to five layers with growth rate of 1.2 and transition ratio 0.77. The mesh consists of 498624 elements and contain 101552 nodes. Under setup section the analysis type was set to steady-state with three distinct domains: air (above fluid), rotor (two rotating domains for impellers) and the stationary fluid domain. The solver control was set to continuity equation class with high resolution advection scheme and first order turbulence numeric. The residual type was set to target value 1E-5. Free surface standard isothermal homogenous k-epsilon turbulence model with scalable wall function was chosen to model the fluid behaviour. The surface tension coefficient was set to 0.072 N/m with continuum surface force and free surface interphase transfer. In boundary conditions the rotating domain has continuous fluid morphology and initial rotational speed of 200 revolutions per minute (Fig. 3).

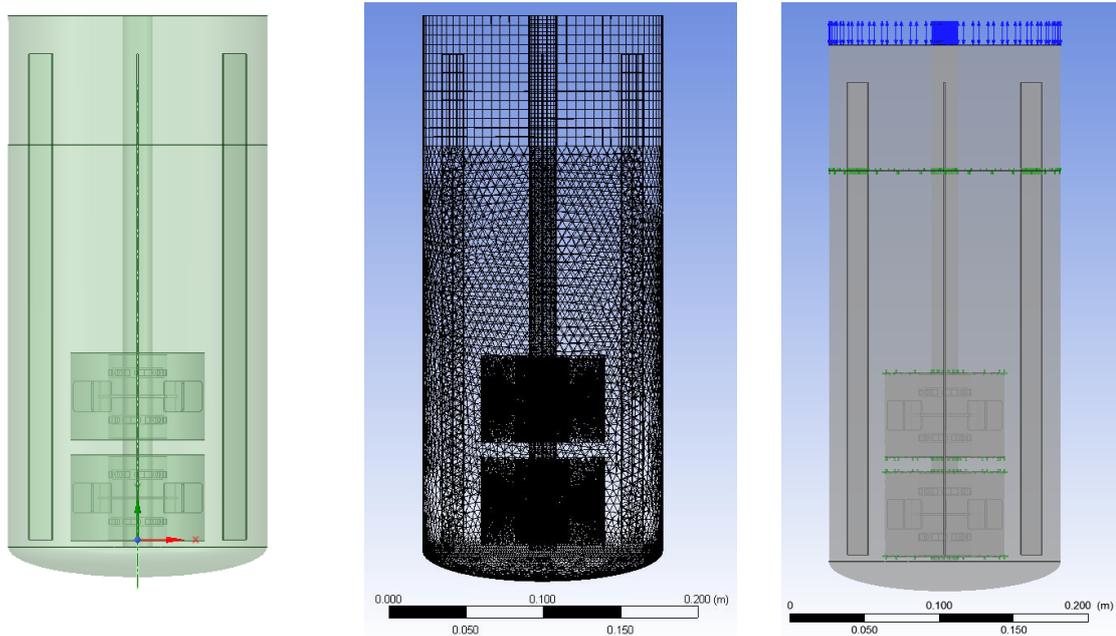


Fig. 2. Geometry, mesh and boundary condition setup profiles.

The model contains two distinct interface regions between: 1) bulk liquid and air above; 2) bulk liquid and rotating domain. The top of the vessel has open boundary condition. The interface model was set to general connection and the frame change (mixing model) was set to frozen rotor. After completing the setup steps the model was executed in platform MPI local parallel run mode using 4 partitions of CPU.

III. RESULTS AND DISCUSSION

The flow velocity profile contains the liquid fraction, section lines and vector field with corresponding velocity values when it is stirred at 200 revolutions per minute. The flow pattern corresponds to the one that radial impellers are expected to create. Higher velocity streams are directed towards the wall forming vortices that travel upwards and downwards creating intense turbulence regions (Fig. 4).

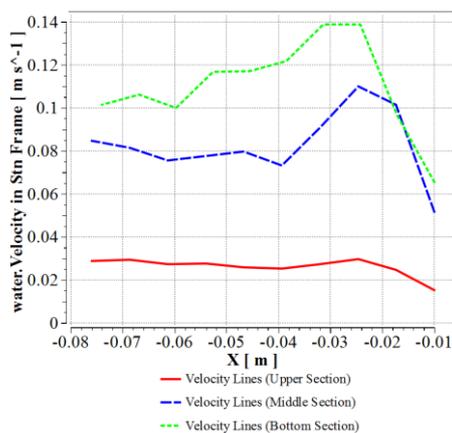


Fig. 3. Liquid velocity along section lines.

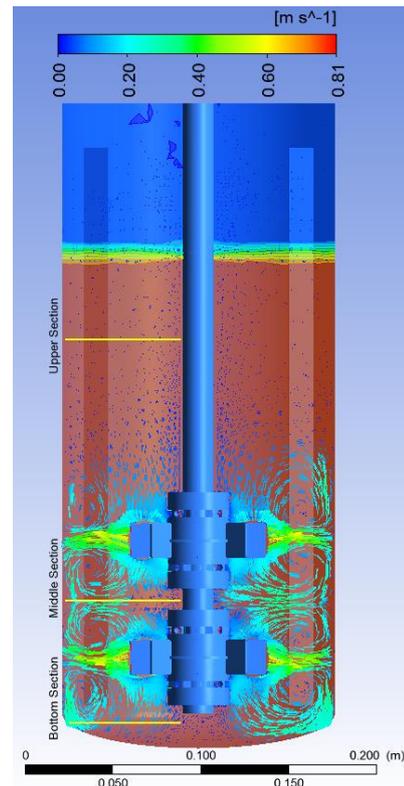


Fig. 4. Velocity profile (XY plane) with vector field and liquid volume fraction (@200 rpm).

Section lines indicate how velocity propagates along the lines (Fig. 3). The upper section has the lowest velocity value which was expected as there is no impeller present suggesting sharp drop of velocity in non-agitated regions.

An interesting region is the thin slit between the shaft and the impeller typical for magnetically coupled rotors (Fig. 5).

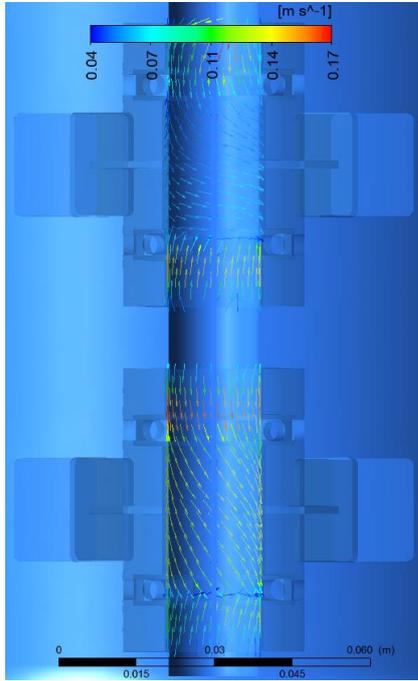


Fig. 5. Thin slit profile between the shaft and the impeller (XY plane, @200 rpm).

In this region flow is predominantly laminar and judging from the vector lines occurs in both directions but mainly downwards suggesting that the intended effect of impeller holes was achieved. The reason for implementing holes was to minimize undesirable flow stagnation. The liquid velocity according to CFD results in this region along the vertical axis is in a range of 0.04 to 0.17 m/s. The slit between the rotor and stationary shaft is 1 mm wide. The rotors are hold in place effectively by magnetic force that originates from a smaller rotating shaft containing neodymium magnets (grade N42, 1.280 T) within the stationary shaft. The height of each rotor is 55 mm. This essentially gives a hollow cylinder with the same height of 55 mm and wall thickness of 1 mm. The cross-section area (A) of such geometric entity (ring) can be determined by equation below:

$$A_{hc} = \pi t(d + t) \quad (1)$$

Where, t is the slit thickness and d is stationary shaft diameter. Substituting the numbers this gives value of $3.14 \cdot 1 \text{ mm} \cdot (18 \text{ mm} + 1 \text{ mm}) = 60 \text{ mm}^2 = 60\text{E-}6 \text{ m}^2$. If liquid velocity in the slit is 0.04 m/s or 40 mm/s according to low end from CFD results (Fig. 5) this would give the volumetric flow rate $0.04 \text{ m/s} \cdot 60\text{E-}6 \text{ m}^2 = 2.4\text{E-}6 \text{ m}^3/\text{s}$. Assuming that the liquid inside reactor is homogenous, the whole bulk of the liquid would pass through the slit opening on average in $5\text{E-}3 \text{ m}^3 / 2.4\text{E-}6 \text{ m}^3/\text{s} = 2083 \text{ s}$ or 35 min. This indicates that each cell has high probability being exposed to strong magnetic fields between NdFeB magnets (grade

N42, 1.28 T, 318 kJ/m^3) during the 35 min mixing time. Cell contact time with 1.28 T could be estimated by dividing rotor height with flow velocity which gives $55 \text{ mm} / 40 \text{ mm/s} = 1.4 \text{ s}$. Studies of magnetic field effects on cells are limited [3], [4], [5] and conclusions vary among researchers.

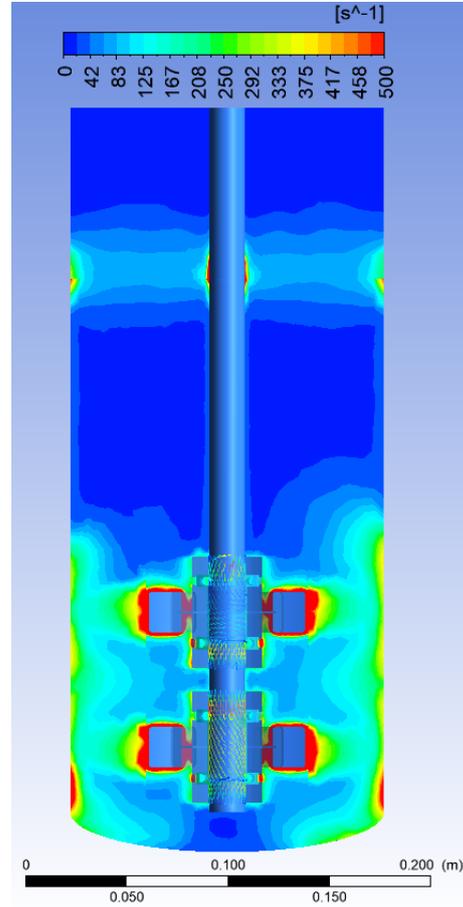


Fig. 6. Turbulence eddy frequency contour (XY plane, @200 rpm).

Turbulence eddy frequency contour (or rotations per second for a typical turbulent eddy) represents high turbulence regions which are most intense in the impeller area and to some extent on the surface where the air and liquid phases come in contact (Fig. 6). The area directly under the impeller and above upper rotor is a lot less turbulent suggesting that mixing in this zone is less effective.

The smallest turbulence length scale η can be determined by the following expression [6]:

$$\eta = \left(\frac{\nu^3}{\varepsilon} \right)^{1/4} \quad (2)$$

Where, ν is the kinematic viscosity of the fluid and ε is the average rate of dissipation of turbulence kinetic energy (per unit mass). The expression above is called Kolmogorov length scale and was first proposed by A. Kolmogorov (1941). This is the smallest scale (eddy size) in turbulent flow. Below Kolmogorov scale turbulent kinetic energy is dissipated as heat and viscosity becomes dominant. This means if eddy size approaches the cell size then significant cell damage is

likely to occur within such medium. Similar theory exists that relates the idea above but uses different variables is called Taylor length scale, which is sometimes called the turbulence length scale [7]. This microscale is named after G.I. Taylor (1915). The Taylor microscale is the intermediate length scale at which fluid viscosity significantly affects the dynamics of turbulent eddies in the flow. This length scale is traditionally applied to turbulent flow which can be characterized by a Kolmogorov spectrum of velocity fluctuations. Below the Taylor microscale the turbulent motions are subject to strong viscous forces and kinetic energy is dissipated into heat.

Reynolds number in a stirred tank can be calculated by the following expression [8]:

$$Re = \frac{\rho ND^2}{\mu} \quad (3)$$

Where, ρ is liquid density (1000 kg/m^3), μ is liquid dynamic viscosity ($0.001 \text{ kg m}^{-1} \text{ s}^{-1}$), D is impeller diameter (0.078 m) and N is impeller angular velocity expressed in s^{-1} ($200/60 \text{ s} = 3.3 \text{ s}^{-1}$). The length scale is related to Reynolds number Re by the following expression [9]:

$$\eta = DRe^{-3/4} \quad (4)$$

From (3) at 200 rpm or 3.3 s^{-1} Re equals 20280 and from (4) η is equal to $46 \text{ }\mu\text{m}$. Most eukaryotic cells are within $10\text{-}20 \text{ }\mu\text{m}$ size range [10] suggesting that mixing regime even at 200 rpm could endanger certain cells but not necessarily destroy them.

In early studies of animal cell cultures, the concern of “shear sensitivity” has been often mentioned [11]. Given the native state of animal cells with all of the mechanical and nutrient support, this concern is understandable, and is further highlighted by lack of cell wall in comparison to the typical microorganisms used in fermentation. The only separation of the animal cell from the surrounding fluid is the plasma membrane consisting of phospholipids, triglycerides, cholesterol, and transmembrane proteins. Therefore, knowing the shear rate is something to consider dealing with delicate cell-cultures. Shear rate is the rate at which a fluid is sheared or “worked” during flow. In more technical terms, it is the rate at which fluid layers move past each other. Shear rate is determined by both the geometry and speed of the flow. CFD indicates shear rate maximum at 1063 s^{-1} which corresponds to $1063 \text{ s}^{-1} \cdot 0.001 \text{ Pa s} = 1.063 \text{ Pa} = 10.63 \text{ dyne/cm}^2$ shear stress (Fig. 7). Some authors report even 2 Pa shear stress as damaging for mammalian cells [12].

Mixing process is associated with agitation input power. Different type of impellers has different power rates at the same angular velocity. Power Number N_p is a dimensionless parameter used for estimating the power input P by the agitating turbine [8].

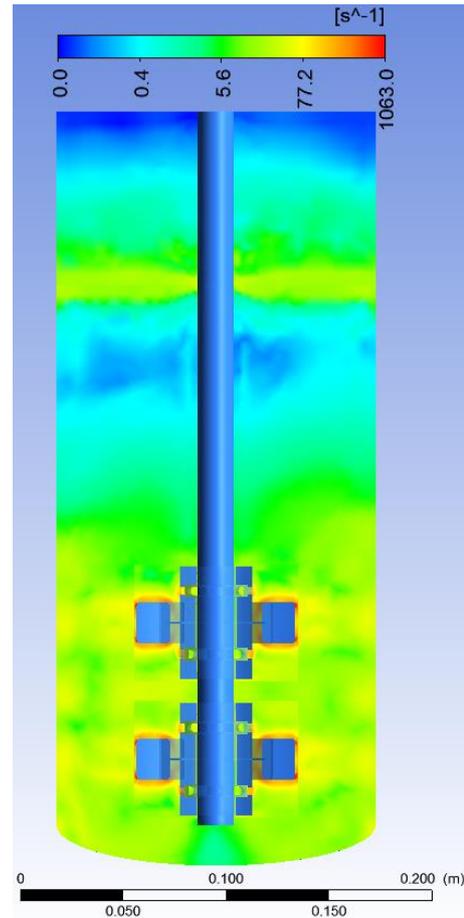


Fig. 7. Shear strain rate (log scale, XY plane, @200 rpm).

The same reactor setup was simulated at 500 and 1000 rpm. The obtained values are represented in TABLE II.

TABLE II.

Revolutions per minute [rpm]	Average stirred tank characteristic variables depending on rpm					
	Re number (x1000)	Turbulence length scale [μm]	Bulk liquid velocity [cm/s]	Thin slit liquid velocity [mm/s]	Eddy frequency in bulk liq. [1/ds]	Shear strain rate @ impellers [1/s]
200	20	46	16	66	35	167
500	51	23	38	99	102	445
1000	101	14	75	180	300	903

Power number is usually plotted vs Reynolds number Re . From our experimental setup the power numbers obtained are shown in TABLE III. Power numbers were calculated by the following equation [8]:

$$N_p = \frac{P}{\rho N^3 D^5} \quad (5)$$

Calculated Re and N_p number are plotted in Fig. 8. The best fit line can be approximated as a power function with negative power: $y=4E7x^{-1.44}$.

TABLE III.

Revolutions per minute [rpm]	Experimental power input with corresponding Re at 100-1000 rpm		
	Input power experimental [W]	Corresponding Reynolds number [-]	Corresponding Power number [-]
100	1.17	10140	87.75
150	1.65	15210	36.56
200	2.37	20280	22.13
250	3.35	25350	16.04
300	4.43	30420	12.27
350	5.90	35490	10.30
400	7.46	40560	8.72
450	9.33	45630	7.66
500	11.05	50700	6.61
550	12.67	55770	5.70
600	14.33	60840	4.96
650	15.59	65910	4.25
700	17.52	70980	3.82
750	20.26	76050	3.59
800	23.37	81120	3.42
850	27.06	86190	3.30
900	29.88	91260	3.07
1000	37.91	101400	2.84

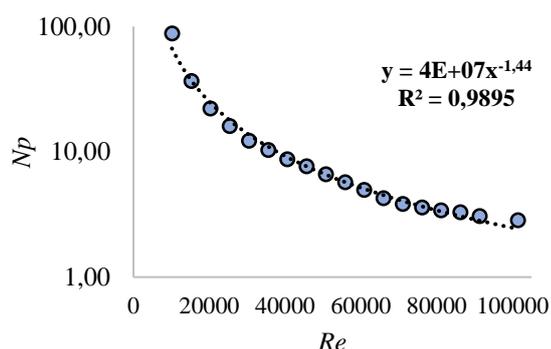


Fig. 8. Power number N_p versus Reynolds number Re : experimental.

The best fit function can be further used for N_p estimation and hence the power requirement, providing the setup has similar aspect ratio as in this research. However, more experimental work could be done to verify the last statement.

IV. CONCLUSIONS

The hydrodynamics of stirred tank bioreactor was studied predominantly using CFD and experimental results. The CFD calculations indicate that flow velocity, turbulent eddy frequency and shear rate are largest in the close proximity of the impeller and smallest underneath the impeller and above upper impeller. According to CFD the impeller holes appear to be beneficial and assist to the flow direction in the thin slit region thus minimizing the flow stagnation in this region. As the mixing rate increases so does the apoptosis risk to cell-based fermentation cultures. 200 rpm regime is considered reasonably safe while 1000 rpm regime is likely damaging to eukaryotic cell cultures due to high shear rate and turbulent length scale

(14 μm) that is close to the cell dimensions. CFD results provide valuable insight into the working bioreactor flow physics in the way that would be impossible or hardly possible with experimental setup. Furthermore, CFD modelling can help in identifying most desirable bioreactor work regime settings to suit the needs of delicate fermentation systems.

ACKNOWLEDGEMENTS

This work has been supported by European Regional Development Fund within the project “Influence of the magnetic field initiated stirring on biotechnological processes” No. 1.1.1.1/16/A/144.

REFERENCES

- [1] K. M. Dhanasekharan and J. L. Kokini, “Design and scaling of wheat dough extrusion by numerical simulation of flow and heat transfer,” *J. Food Eng.*, vol. 60, no. 4, pp. 421–430, 2003.
- [2] J. Liang and E. W. C. Lim, “Evaluation of RANS Turbulence Models, les and des for CFD Simulations of Bubbling, Turbulent and Core-Annular Fluidization,” *J. Chem. Eng. Japan*, vol. 51, no. 8, pp. 646–663, 2018.
- [3] F. T. Hong, “Magnetic field effects on biomolecules, cells, and living organisms,” *BioSystems*, vol. 36, no. 3, pp. 187–229, 1995.
- [4] K. Fijałkowski, P. Nawrotek, M. Struk, M. Kordas, and R. Rakocz, “The effects of rotating magnetic field on growth rate, cell metabolic activity and biofilm formation by staphylococcus aureus and Escherichia coli,” *J. Magn.*, vol. 18, no. 3, pp. 289–296, 2013.
- [5] M. Konopacki and R. Rakoczy, “The analysis of rotating magnetic field as a trigger of Gram-positive and Gram-negative bacteria growth,” *Biochem. Eng. J.*, vol. 141, no. 15, pp. 259–267, 2019.
- [6] C. Johnson, V. Natarajan, and C. Antoniou, “Verification of energy dissipation rate scalability in pilot and production scale bioreactors using computational fluid dynamics,” *Biotechnol. Prog.*, vol. 30, no. 3, pp. 760–764, 2014.
- [7] S. Tavoularis and U. Karnik, “Further experiments on the evolution of turbulent stresses and scales in uniformly sheared turbulence,” *J. Fluid Mech.*, vol. 204, pp. 457–478, 1989.
- [8] J. A. Sánchez Pérez, E. M. Rodríguez Porcel, J. L. Casas López, J. M. Fernández Sevilla, and Y. Chisti, “Shear rate in stirred tank and bubble column bioreactors,” *Chem. Eng. J.*, vol. 124, no. 1–3, pp. 1–5, 2006.
- [9] V. N. Vlachakis, P. P. Vlachos, D. P. Telionis, M. R. Brady, and R. H. Yoon, “Turbulence Characteristics in a Rushton Stirring Vessel: A Numerical Investigation,” in *ASME 2006 2nd Joint U.S.-European Fluids Engineering Summer Meeting Collocated With the 14th International Conference on Nuclear Engineering*, 2008, pp. 19–26.
- [10] D. A. Guertin and D. M. Sabatini, “Cell Size Control,” *Encyclopedia of Life Sciences*. pp. 1–10, 2005.
- [11] W. Hu, C. Berdugo, and J. J. Chalmers, “The potential of hydrodynamic damage to animal cells of industrial relevance: Current understanding,” *Cytotechnology*, vol. 63, no. 5, p. 445, 2011.
- [12] T. Tanzeglock, M. Soos, G. Stephanopoulos, and M. Morbidelli, “Induction of mammalian cell death by simple shear and extensional flows,” *Biotechnol. Bioeng.*, vol. 104, no. 2, pp. 360–370, 2009.