To mix effectively and not kill: fluid dynamics and energy dissipation rate distribution in AppliFlex bioreactors

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ABSTRACT

High shear rates and turbulence levels are known to have an influence on the viability of mammalian cells, which often leads to lower yields in bioreactor cultivations [1].

Impeller tip speed and a constant power per volume (P/V) input are parameters often used for scaling up/down shear sensitive bioreactor cultivations [2]. Impeller tip speed (m s-1) is commonly used to quantify the maximum shear forces in stirred tank bioreactors. However, it fails to capture the mixing conditions in the reactor. Also, it does not consider the residence time of the cells in which they will be exposed to this local high shear. By contrast, volumetric power input (W m-3) only reflects the total average energy dissipated inside the reactor, but not the local distribution.

Turbulent local energy dissipation rate (EDR; W m-3) offers several advantages over P/V and tip speed: (i) it is dependent on only fluid and flow properties, (ii) it accounts for both shear and extensional flow in a bioreactor, and (iii) it provides local information that can help to determine the spatial distribution of turbulence in the reactor [3].

In this work, EDR has been used to quantitatively evaluate different 3 impeller designs available for AppliFlex ST bioreactors (Getinge Applikon, The Netherlands). A computational fluid dynamics (CFD) approach was followed to determine the circulation patterns in these bioreactors and to calculate the total and local EDR. The results show that the helical impeller has lower local maximum EDR (EDRmax) compared to marine and hydrofoil impellers at the same overall P/V. This suggests that this impeller is more adequate for cultivations of shear-sensitive mammalian cell lines.

MATERIALS AND METHODS

1 The geometries of the marine, hydrofoil and helical impellers available in the AppliFlex ST bioreactors (Getinge Applikon, The Netherlands) were used, with a liquid working volume of 400 mL (Figure 1).
2 No dispersed gas phase was considered, as it was assumed that aeration was only via headspace and not by sparging. This strategy is appropriate for low cell concentration cultivations, or cultures of cell lines with low oxygen requirements.
3 COMSOL 5.4 was used for all CFD simulations, using a rotating domain approach and a realizable k-ε turbulent model for the liquid phase.
4 Mesh independence was evaluated for shear stress and total power input varying the maximum mesh element size from 0.1 to 1.0 cm.
5 Water rheological properties were chosen at 37°C to reflect the temperature at which mammalian cell cultures are performed.
6 Post-processing of the CFD results was performed in MATLAB to interpolate shear rates, liquid velocities, and the turbulent component of the EDR$_{(\epsilon_{tur})}$ to a 0.1 mm x 0.1 mm x 0.1 mm grid. Local total EDR$_{(\epsilon)}$ was calculated for each element (Figure 2).
7 Total power input was calculated using the impeller torque, and validated integrating the local EDR over the complete reactor volume.

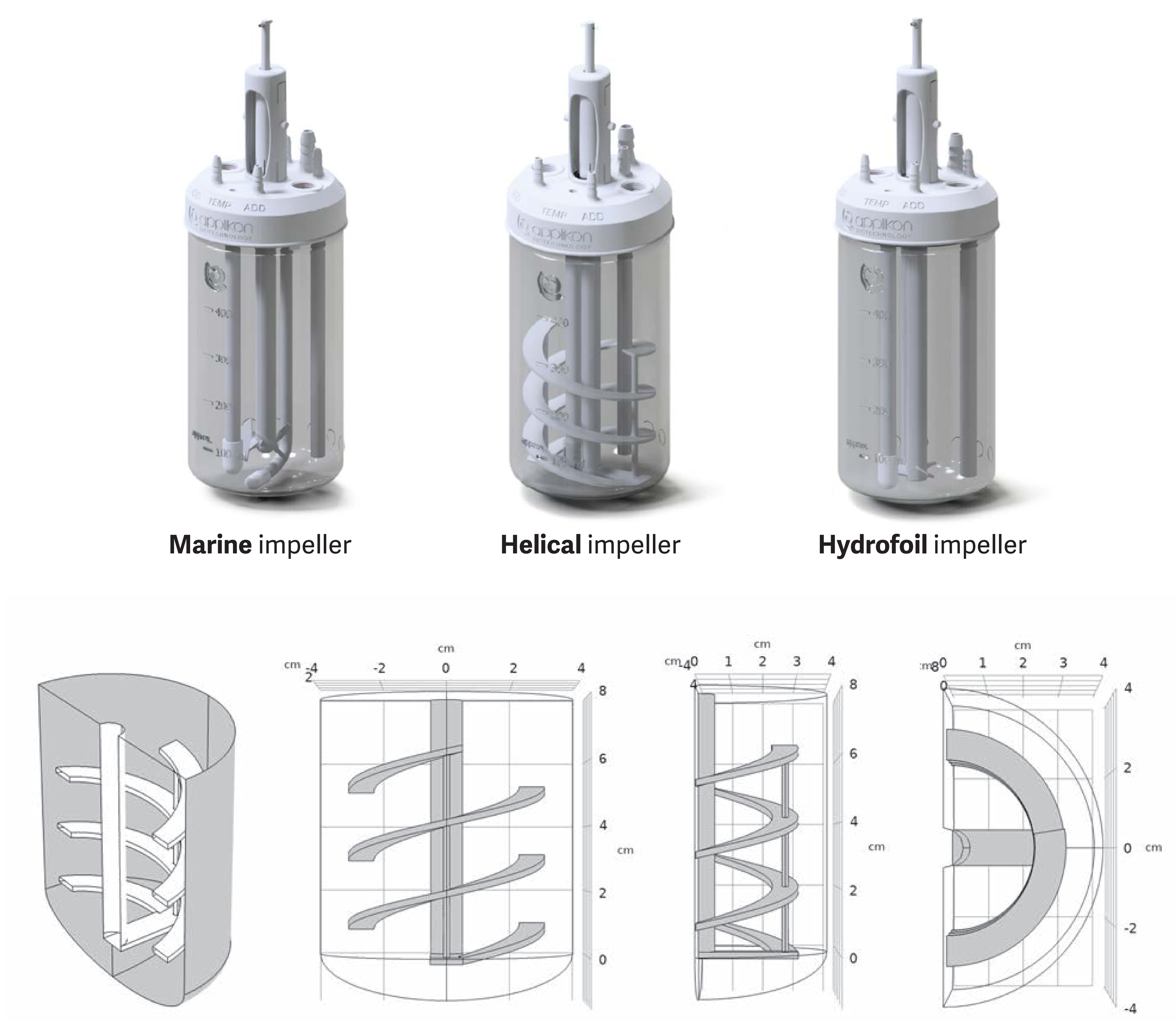

Figure 1 | (Top) AppliFlex 3D-printed bioreactors with the three impeller studied. (Bottom) Example of the geometry implemented in COMSOL for the CFD simulation. In the case of a helical impeller, a half-rotation symmetry was used, while a 1/3-rotation symmetry was used for the marine and hydrofoil impellers.

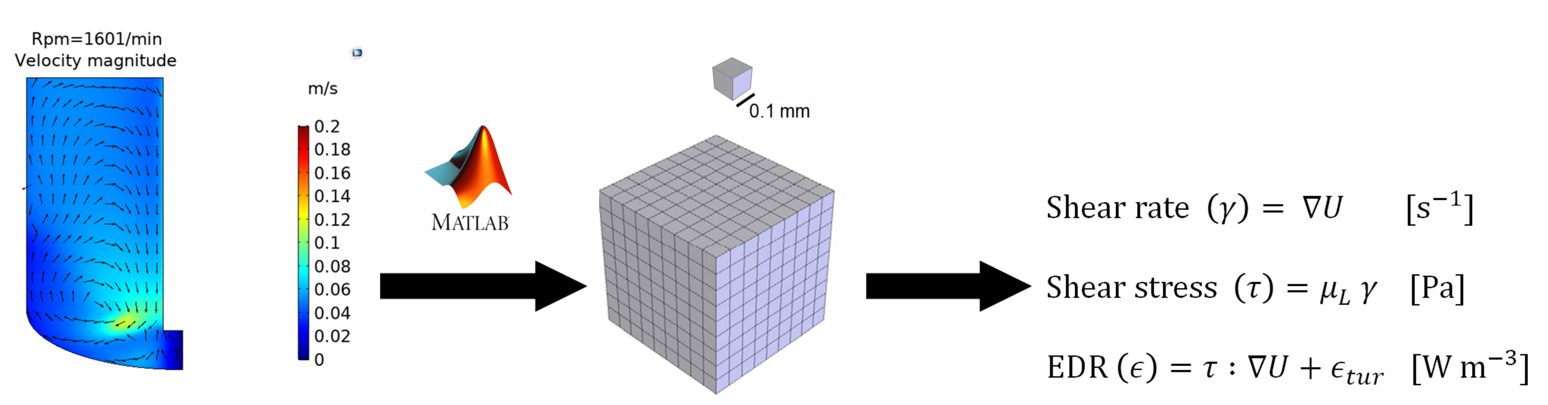

Figure 2. Approach for the calculation of local EDR. Results from the CFD simulations are interpolated using MATLAB to a 3D grid of 0.1 x 0.1 x 0.1 mm elements, and used to calculate local shear and EDR.

RESULTS AND DISCUSSION

Impeller geometries influence the hydrodynamic conditions in the reactor, which would have an effect on the distribution of EDR. To evaluate this, we calculated histograms of the EDR distribution for the three impellers using the same tip speed (Figure 3).

These results suggest that **hydrofoil and marine impellers have a less homogeneously distributed turbulent energy dissipation rate compared to the helical impeller,** with a few volume elements contributing as much to the mean EDR as a large number of low EDR volume elements.

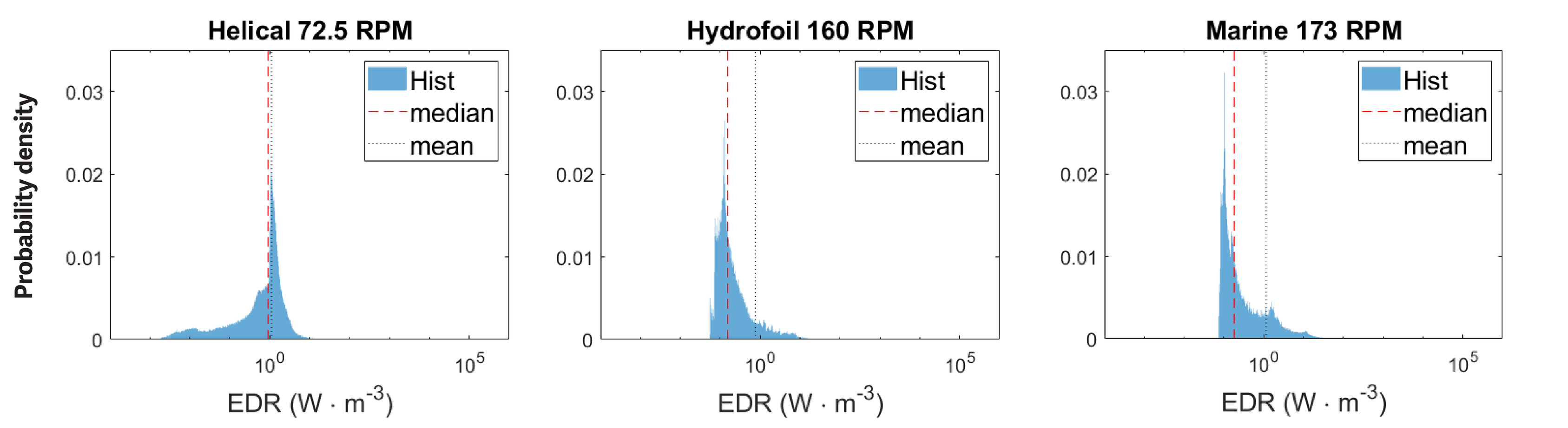

Figure 3. For the same tip speed (23 cm s-1), the helical impeller (left) has a positive-skewed EDR distribution, while the hydrofoil (middle) and marine (right) impellers have a negative-skewed distribution.

EFFECT OF TIP SPEED ON LOCAL EDR

To evaluate the effect of tip speed on the EDR (mean and max), multiple CFD simulations were performed for the three impeller geometries (Figure 4). Results show that for all tip speeds, **the helical impeller has lower values of local maximum energy dissipation rate (EDRmax) compared to the other two geometries.**

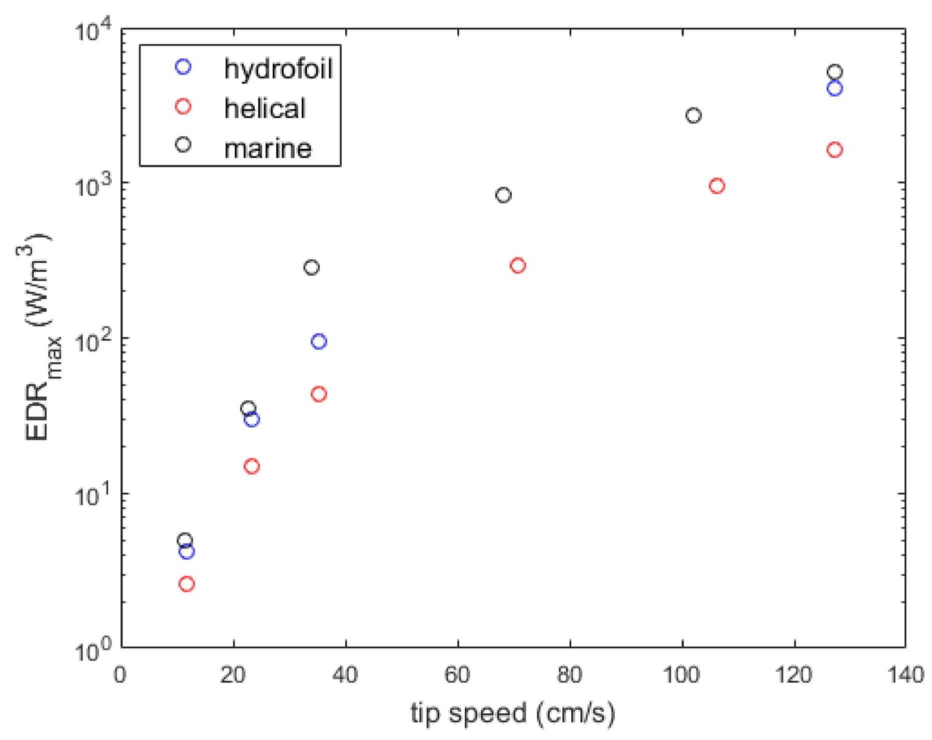

Figure 4. The correlation between tip speed and EDRmax is impeller specific. Hydrofoil and marine impeller behave similarly, while the helical impeller has a lower local maximum energy dissipation rate for the whole range of tip speeds that was studied.

CORRELATION BETWEEN MAXIMUM LOCAL EDR AND TOTAL DISSIPATED ENERGY IN THE REACTOR

Having a lower EDRmax could protect the cultured cells from excessive turbulence. One way to achieve this is to have lower tip speeds. However, this would also lead to a badly-mixed reactor. The total power per volume is a common indicator of the quality of mixing in a stirred tank. Figure 5 shows that for the same mean EDR (analogue to total P/V), the helical impeller has lower EDRmax. **This suggests that for the same mixing level, the helical impeller results in lower local high turbulence areas.**

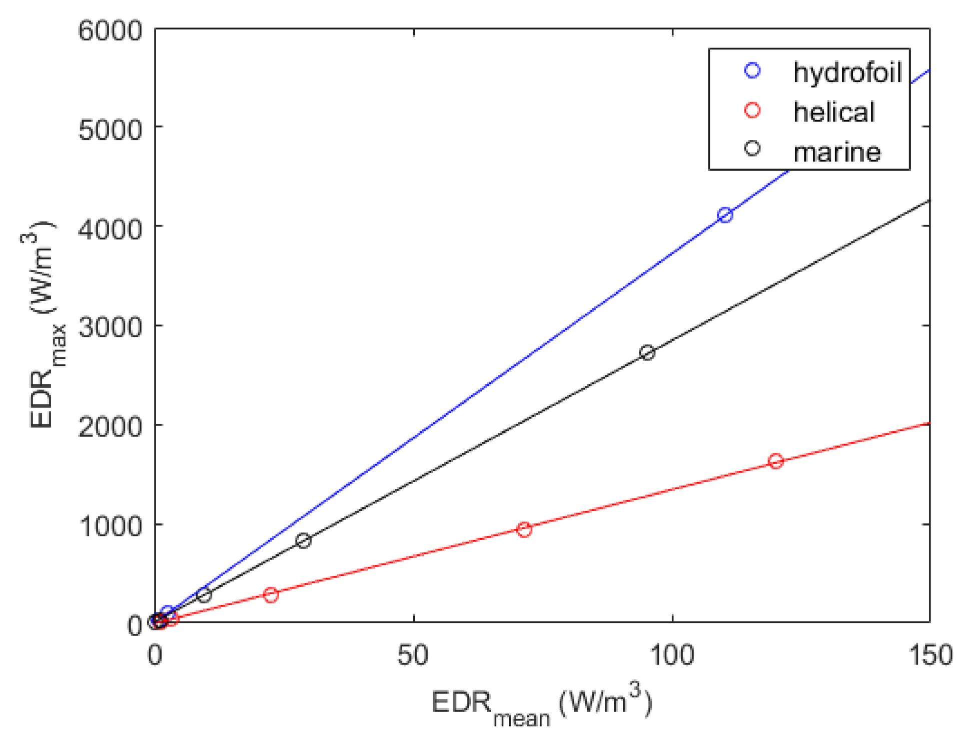

Figure 5. The correlation between total P/V (EDRmean) and EDRmax is also impeller specific. With the same input of mixing power, lower local maximal EDRs will be obtained when using the helical impeller.

References

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CONCLUSIONS

- CFD simulations can be used to study the local distribution of turbulent energy being dissipated in stirred tank reactors.
- The helical impeller generates the lowest EDRmax values at the same P/V input, compared to the marine and hydrofoil impellers. This is also true if the same tip speed is used to compare the three impellers.
- The evaluated helical impeller geometry is a better design for shear sensitive processes, having the most homogeneous EDR distribution in the bioreactor.

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