Review on the Impact of Impeller-induced Hydrodynamics on Suspension Cell Culture for Production of Biopharmaceuticals

Ralf Pörtner*, Fabian Freiberger, and Johannes Möller

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Dedicated to Prof. Dr.-Ing. Peter Czermak on the occasion of his 65th birthday

Hydrodynamic effects continue to play a crucial role in the design and operation of bioreactors for cultivating mammalian cells. Despite intensive studies in the past the link between hydrodynamics and biological process behaviour is still not fully understood. Common methods to describe dependencies, which relay mostly on averaged hydrodynamic parameters, does not seem to be suitable, as they do not incorporate the hydrodynamic heterogeneity. Within this review first a brief introduction to hydrodynamic effects is given, followed by an evaluation of common process design approaches with respect to hydrodynamic effects and recommendations for process development.

Keywords: Bioreactor characterization, Computational fluid dynamics, Energy dissipation rate, Kolmogorov length scale, Mammalian cell culture, Operational space, Process development, Shear effects

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1 Introduction

Hydrodynamic effects continue to play an elemental role in the design and operation of bioreactors for cultivating mammalian cells [1, 2]. A basic distinction can be made between (1) applications in tissue engineering or regenerative medicine including cell and gene therapy, and (2) production of biopharmaceuticals. With respect to (1) investigation of shear-induced effects focusses mainly on cellular physiology and differentiation on a sub-lethal level (e.g., mechano-stimulation) [3–5], whereas in (2) sub-lethal and lethal hydrodynamic effects in culture systems from lab to production scale and the integration of these effects into design strategies are considered [6–8]. This review will address mainly aspects related to production of biopharmaceuticals.

In general, a huge know-how on the effects of hydrodynamics on cell lines used in production of biopharmaceuticals is available due to intensive studies [1]. Besides basic results in model systems with defined flow conditions, especially hydrodynamic effects in sparged stirred tank reactors (STRs) have been considered, as these are the working horse of industrial cell culture technology [1, 9]. Stirring and sparging are essential to provide a homogeneous mixing and an appropriate supply of nutrients and oxygen to the cells [10]. But as both induce hydrodynamic stress that could damage mammalian cells or affect the performance of the cells [11], these effects must be taken into account when designing the reactors and selecting process parameters such as stirrer speed or aeration rate [9].

Cell lines used for routine manufacturing of biopharmaceuticals, mostly CHO cell lines, are regarded as robust in typical bioprocessing applied today and are cultivated in suspension cultures [1]. Suitable operation conditions have been worked out for industrial scale processes. However, Chalmers et al [2] state that as the achievable final cell concentrations and product titers continue to increase, significantly higher levels of mixing and aeration are required causing damaging hydrodynamic effects.

Although the shear sensitivity of cell cultures is generally taken into account in reactor design [12], the question
"*How can hydrodynamic-induced damages of cells be considered in process design?*"
has not finally been answered. Especially the exact relationship between hydrodynamics in bioreactors and the effect on cell behaviour is not fully understood. Therefore, the design of biopharmaceutical manufacturing processes relies to a huge extent on empirical values or correlations and rules of thumb. Usually, only system-averaged hydrodynamic parameters are considered to predict the growth and

[1]Prof. Dr.-Ing. Ralf Pörtner
https://orcid.org/0000-0003-1163-9718 (poertner@tuhh.de),
[1]Dr.-Ing. Fabian Freiberger, [1]Dr.-Ing. Johannes Möller
https://orcid.org/0000-0001-9546-055X
[1]Hamburg University of Technology, Institute of Bioprocess and Biosystems Engineering, Denickestraße 15, 21073 Hamburg, Germany.

production behaviour of cell cultures, e.g. the average power input or mixing time in a bioreactor. The heterogeneities of the fluid seem to be given little attention, even if these can be assessed nowadays by computational fluid dynamics (CFD) [13–15].

In this review, the above-mentioned questions will be addressed with focus on hydrodynamic effects in stirred tank reactors caused by the stirrer, as stirring is the main source of hydrodynamic cell damage throughout process scales. Damage to cells caused by gas bubbles is obviously relevant as well [16, 17]. But as has been shown by Henzler and Kauling [18], the mechanical stress caused by bubbles decreases significantly with scale. Therefore the effects of bubbles have not been considered here. For further review on effects caused by bubbles see [2]. Furthermore, a brief introduction to hydrodynamic effects is followed by an evaluation of common process design approaches with respect to hydrodynamic effects and recommendations for process development.

2 Hydrodynamic-induced Effects on Cells – Link Between Hydrodynamic and Cellular Effects

Various lethal and sub-lethal effects of hydrodynamics on mammalian cells used for production of biopharmaceuticals are described in the literature (for Review see [1, 2]). Whereas earlier studies addressed mainly adherent cells with respect to growth on microcarriers, the focus shifted to suspension cells (e.g., hybridoma, CHO), as these are more relevant for the industrial manufacturing of biopharmaceuticals. Furthermore, a distinction can be made between studies in model systems with defined hydrodynamic conditions (e.g., flow chambers with defined wall shear stress) and those in real life bioreactor systems, mostly stirred tanks. Fundamental studies in model systems are helpful for understanding the underlying mechanisms, but transfer to production scale is still difficult. Bioreactor systems are too complex for fundamental studies, but essential to work out appropriate operation ranges without damaging effects.

Hydrodynamic effects on cells are often related to "shear stress". Shear stress is defined as the gradient of the velocity perpendicular to the flow multiplied with dynamic viscosity η of the fluid. This definition can be applied to define the shear stress in model systems, e.g., laminar flow between parallel plates to calculate the wall shear stress acting on adherent cells. For more complex flow situation as in STRs, normal stress must be considered as well, which is defined as the gradient of the velocity in the direction of the flow multiplied with the dynamic viscosity. To add further complexity, the hydrodynamic conditions change locally and with time.

Due to the described complexity it is still not possible to link hydrodynamic effects in complex flow to representative values of shear stress [1]. Therefore, for this review the term "hydrodynamic effects" is used rather than the term "shear stress effects". A parameter that is often used to characterise hydrodynamic effects is the energy dissipation rate *EDR*, especially to characterise mixing effects in stirred tanks [19]. The *EDR* can be expressed mathematically for an incompressible Newtonian fluid as the ratio between a stress tensor and a velocity gradient. For complex flow, and turbulent, or near-turbulent conditions, such as in a typical STR, first principles solutions are not possible, but can be accessed by computational fluid dynamics (CFD) (see below).

In the following two terms will be used to quantify the Energy Dissipation Rate *EDR*: (1) the volume specific energy dissipation rate *vEDR* [$W\,m^{-3}$] in case of locally *EDR*'s determined via CFD or in case of devices other than STR's, (2) the average volume specific energy dissipation rate $vEDR_{av}$ [$W\,m^{-3}$] equal to the (average) volume specific power input P/V [$W\,m^{-3}$] for STR's. In the latter case the mean power input P can be estimated by the Newton/power number for the specifically used impeller [20].

Tab. 1 provides a survey on reported hydrodynamic effects for suspension cultures in stirred lab-scale culture systems. Effects of bubble aeration have not been considered specifically here.

Sieck et al. [9] as well as Chalmers et al. [1] related sub-lethal physiological responses critical to bioprocesses and lethal effects (necrosis including LDH release) of hydrodynamic forces on cells and linked these to various levels of the volume specific energy dissipation rate within the respective culture systems. These data have been summarized in Fig. 1, supplemented with the range of maximal values reported for STRs. On the one hand it is confirmed that specific effects depend on the cell line/clone and/or the cultivation system. On the other hand, it becomes obvious that most non-lethal effects determined mostly in model systems are still above typical operating conditions in commercial STRs. Even maximal values reported for STRs are below these threshold values for lethal effects. Chalmers et al. [1] conclude that these non-lethal effects "at this point have more "academic" than "industrial" significance".

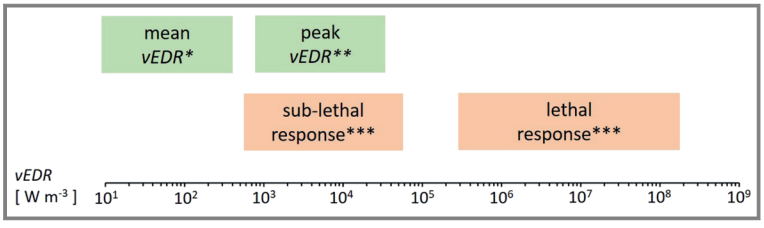

Figure 1. Comparison of reported ranges for mean and peak volume specific energy dissipation rate *vEDR*'s as well as ranges for sub-lethal and lethal cellular response's. Data mostly for established cell lines (e.g., hybridoma and CHO). * Stirred tank bioreactors (data see Tab. 1, mean *vEDR* = *P/V*), ** Stirred tank bioreactors, mostly CFD (data from [21, 30, 38]), *** Model systems with defined flow conditions (cited by [2, 9]).

Table 1. Survey on reported hydrodynamic effects for suspension cultures in stirred lab-scale culture systems without considering effects of bubble aeration.

Cell type, Medium	Cultivation system	Criterion	Parameter	Operation range	Ref.
Hybridoma	1 L stirred tank	VCD	Stirrer speed	No damaging effect between 100 and 45 rpm	Cited by [35]
Hybridoma, serum-containing medium	Stirred tank, gas-free	Cell death	$vEDR_{av}$	Damaging Threshold Values < 350 W m^{-3}	Cited by [11]
Sp2/0 mouse myeloma, serum-free medium	Spinner flask	Cell death	Shear rate at tip	Damaging threshold values < 1.2 s^{-1}	[36]
	3 L Stirred tank		Stirrer speed	Damaging threshold values < 90 rpm	
Murine hybridoma, RPMI 1640 supp. with 10 % FBS	2 L stirred tank, 6-blade radial impeller	Growth rate	$vEDR_{av}$	Increasing growth rate 4 – 780 W m^{-3}	Cited by [20]
				Decreasing growth rate > 780 W m^{-3}	
NS1-hybridoma, medium supp. with 5 % calf serum	Stirred tank, 2 marine propellers	Growth rate	$vEDR_{av}$	Increasing growth rate 1.5 W m^{-3}	Cited by [20]
				Increased death rate > 320 W m^{-3}	
Hybridoma, RPMI supp. with 10 % serum	750 mL stirred tank, 4-blade radial impeller	Growth rate	$vEDR_{av}$	Increasing growth rate up to 88 W m^{-3}	Cited by [20]
				Increased death rate > 410 W m^{-3}	
AGE1.HN, chemically defined medium, serum-free	200 mL stirred tank, conical, 3-blade marine propeller	Growth rate	$vEDR_{av}$	Constant growth rate between 3 and 54 W m^{-3}	[20]
				Decreasing growth rate > 54 W m^{-3}	
AGE1.HN, chemically defined medium, serum-free	500 mL stirred tank, 3-blade radial-discharging impeller	Growth rate	$vEDR_{av}$	Constant growth rate between 138 and 550 W m^{-3}	[20]
				Decreasing growth rate > 550 W m^{-3}	
AGE1.HN, chemically defined medium, serum-free	500 mL stirred tank, 3-blade radial-discharging impeller	Growth rate	$vEDR_{av}$	Increasing growth rate between 40 and 420 W m^{-3}	[20]
				Decreasing growth rate > 420 W m^{-3}	
CHO	2 L stirred tank	Antibody productivity	$vEDR_{av}$	Decreasing antibody productivity and glycosylation effect > 400 W m^{-3}	[9]
CHO-DP12, chemically defined medium, serum-free	200 mL stirred tank, conical, 3-blade marine propeller	Viable cell density	$vEDR_{av}$	Increasing VCD between 2.4 and 107 W m^{-3}	[21]
				Decreasing VCD > 107 W m^{-3}	
		Antibody time space yield		Increasing STY between 2.4 and 47 W m^{-3}	
				Decreasing STY > 47 W m^{-3}	

Table 1. Continued.

Cell type, Medium	Cultivation system	Criterion	Parameter	Operation range	Ref.
CHO-DP12, chemically defined medium, serum-free	200 mL stirred tank, conical, three stages (3-blade marine propeller + 2 rushton turbines)	Viable cell density	$vEDR_{av}$	Increasing VCD between 73 and 1750 W m^{-3}	[21]
				Decreasing VCD > 1750 W m^{-3}	
		Antibody time space yield		Constant STY between 73 and 4740 W m^{-3}	
HEK293, HEK FreeStyle™ 293-F	4 L stirred tank, 2-blade segmented impeller	VCD	$vEDR_{av}$	Increasing VCD between 63 and 233 W m^{-3}	[37]
				Decreasing VCD > 233 W m^{-3}	

Nevertheless, for operating STRs in the biopharmaceutical sector it is indispensable to design a "safe" operation range with respect to relevant process parameters such as stirrer speed or volume specific power input. According to Chalmers [1] and Fig. 1 the mean volume specific energy dissipation rate (equal to the volume specific power input) should be between approx. 10 and several hundred W m^{-3}. This range has been indicated by others as well [11]. But it is not clear to what extent this range depends on the cell line or reactor system or type of impeller used. Despite the huge number of studies addressing hydrodynamic effects on cells only very few provide data in this respect. In Fig. 2, reported data on the relationship between maximal cell specific growth rate and average volume specific energy dissipation rate $vEDR_{av}$ for different lab-scale stirred bioreactor systems and cell lines are summarized. First of all, it is confirmed that hydrodynamic effects depend on the cell line and the type of impeller. Obviously all three cell lines can be cultivated within a wide operation range, before the growth rates decline significantly. Of the three cell lines examined, the CHO cell line appears to be the most robust. Furthermore, it is obvious that the cells can be cultivated much more stably in multi-stage stirring systems. For CHO cells $vEDR_{av}$ values above 1000 W m^{-3} could be applied without significant decrease of the growth rate. This effect can be explained by comparing the mean and maximum values of energy dissipation. Freiberger et al. [21] provided data for the bioreactor equipped with (1) a one stage impeller (three-blade marine impeller) and (2) a three-stage impeller (1× three-blade marine impeller and 2× Rushton turbine), determined by CFD (Fig. 3). For the one stage system, the maximal volume specific energy dissipation rate determined from averaged slize plots along the reactor height ($vEDR_{SP,max}$) increase stronger as for the three stage system. Furthermore, the ratio between maximal ($vEDR_{SP,max}$) and mean volume specific energy dissipation rate determined from averaged slize plots (mean $vEDR_{SP}$) is significantly higher for the one stage system and remains on this high level with increasing average volume specific energy dissipation rate $vEDR_{av}$. For the three stage system the maximum values are significantly lower compared to the one stage system and the ratio between maximal and mean value decreases with increasing mean value.

Even if the provided data have been obtained in small scale lab systems only, they give at least some hints, why hydrodynamic effects seem to be at least different on small and large scale STRs, respectively, as large scale STRs are mostly designed as multi-stage systems. With respect to process design it becomes obvious, that $vEDR_{av}$ = P/V does not seem to be an appropriate parameter to predict the impact of hydrodynamics in different bioreactor systems and scales (see next section).

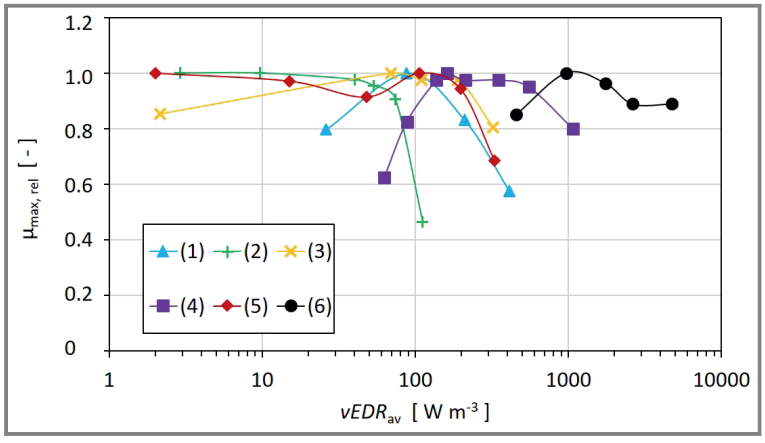

Figure 2. Comparison of reported data on the relationship between maximal cell specific growth rate and the average volume specific energy dissipation rate $vEDR_{av}$ [W m^{-3}] for different lab-scale stirred bioreactor systems and cell lines. Maximal cell specific growth rate is given as relative value ($\mu_{max,rel.}$), growth rate for a certain $vEDR_{av}$ related to the maximal value determined. Maximal cell specific growth rates : hybridoma cell line – 1.4 d^{-1} [39], cell line AGE1.HN – 0.4 d^{-1} [20], CHO cell line – 0.8 d^{-1} [21].
(1) 1 stage, rushton turbine, 0.75 L, hybridoma [39]; (2) 1 stage, 3-blade marine propeller, 0.2 L, AGE1.HN [20]; (3) 2 stage, 4-pitch-blade turbine, 1.0 L, AGE1.HN [20]; (4) 2 stage, 6-blade radial-discharging impeller (Rushton), 1.2 L, AGE1.HN [20]; (5) 1 stage, 3-blade marine propeller, 0.15 L, CHO [21]; (6) 3 stage, 1x 3-blade marine propeller, 2x rushton turbine, 0.15 L, CHO [21]

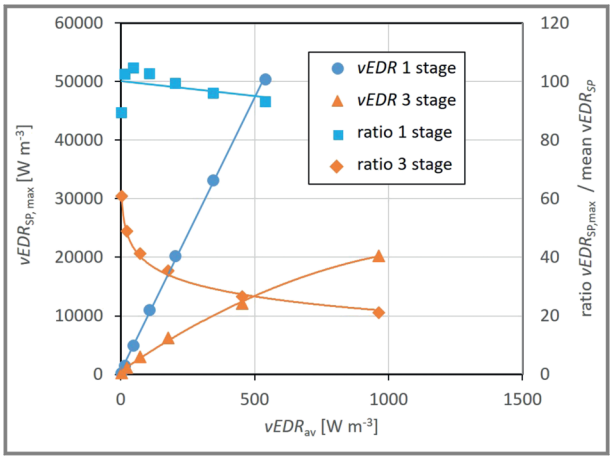

Figure 3. Comparison of the maximal volume specific energy dissipation rate ($vEDR_{SP,max}$) and the mean volume specific energy dissipation rate (mean $vEDR_{SP}$), both from averaged slice plots along the reactor height determined via CFD, for a 1 stage impeller (three-blade marine impeller) and a three stage impeller (1x three-blade marine impeller and 2x Rushton turbine). Bioreactor: 0.15 L (data from [21]).

Furthermore, Fig. 2 indicate that for some impeller configurations a significantly reduced growth has to be expected at low power input, respectively low stirrer speed. This seems to be mainly due to insufficient mixing and is more pronounced for multi-stage-systems. The extent to which oxygen limitation occurs locally due to insufficient mixing leading to decreased growth rate cannot be assessed based on the available data.

Overall it becomes clear, that thorough investigations are required to work out an appropriate operation range. With respect to scale-up, scale-down models of the hydrodynamic stress present in large scale production bioreactors seem to be an appropriate approach to investigate the performance of cells under simulated production scale conditions [9] (compare Sect. 4).

3 Evaluation of Common Process Design Approaches with Respect to Hydrodynamic Effects

So far, hydrodynamic effects have been discussed, but no attention has been given to how relationships between hydrodynamic and cellular parameters (e.g., growth rate, death rate or productivity) can be described quantitatively and how this can be incorporated into a reactor design and scale-up. When developing industrial processes, mostly empirical correlations, experience values and heuristics are used. A common method in process design and scale-up is, for example, keeping the reactor geometry or a certain process parameter constant between different scales. Typically, the average volume specific energy dissipation rate (equal to the volume specific power input), the stirrer tip speed, mixing time, Reynolds-number or combinations are considered in this respect [15, 22–24].

Of the parameters mentioned above, the average volume specific energy dissipation rate in particular is used for scale up. For example, for CHO cultivation processes in industry, an average volume specific energy dissipation rate between 10 W m^{-3} and 50 W m^{-3} is often chosen, while for cell culture processes in general even average volume specific energy dissipation rate of 10 W m^{-3} to 250 W m^{-3} or 50 W m^{-3} to 1000 W m^{-3} can be found (compare as well in Fig. 2). [25, 26] But as has been shown in the previous section, a clear relationship between the average volume specific energy dissipation rate and cellular parameters reflecting hydrodynamic effects can hardly be found. The fact that the criterion still appears suitable for scale-up is probably due to the stability of the cells over a wide range as shown in Fig. 3.

Freiberger et al. [21] examined, if hydrodynamic effects can be related to the maximal energy dissipation rate, but again this parameter was not sufficient as indicator for the impact of hydrodynamics on the cells.

A frequently discussed criterion for assessing the influence of hydrodynamic forces resulting from stirring on cell growth is based on the Kolmogorov length theory. This theory states that the smallest turbulence eddies in the reactor have a cell-damaging effect as soon as they are smaller than the size of the cells or a certain fraction of them or the size of the microcarriers in case of adherent cell growth. This theory was first elaborated for microcarrier culture and later expanded to suspension cells [16, 27–29]. Due to practical reasons, the energy dissipation rate required for calculation of the Kolmogorov length scale is usually determined as the average volume specific energy dissipation rate, which can be easily determined from the power input. For microcarrier cultures the concept seems to work quite well, probably due to the large size of the microcarriers (approx. 100–200 µm) and the narrow size distribution. For suspension cells, which are much smaller (average 10–20 µm) with a large size distribution, this approach is controversial. In the literature, connections between the growth behaviour of suspended cells and the Kolmogorov length scale are described, in which the Kolmogorov length and the cell size are related. Negative effects on cell growth were described in cases in which the Kolmogorov length was already smaller than half the cell size. In addition, cell size distributions were not taken into account, nor was the Kolmogorov length calculated based on local maxima of the energy dissipation rate.

Freiberger et al. [21] calculated the Kolmogorov eddy length scale for suspension CHO-cultures in the form of distributions instead of one fixed length for the whole bioreactor due to strong hydrodynamic heterogeneities in the cultivation systems. In addition, cell size distributions instead of a mean cell size were considered. The resulting critical energy dissipation rate distribution, calculated from cell size distributions, was compared to the averaged and maximum energy dissipation rate as well as to the maxima

from slice plots and volumetric distributions of the energy dissipation rate, all from CFD simulations. It was found that the Kolmogorov length scale is not suitable to describe a link between hydrodynamics and cell damage, if the averaged or maximum energy dissipation rate is used since they do not correlate with any biological observations.

Therefore, Freiberger et al. [21] extended the working hypothesis that the connection between hydrodynamics and the cellular response of the process can be attributed to a critical volume fraction of the cultivation system. A similar approach has been suggested by [30, 31]. Negative effects on cell growth and antibody production should therefore occur if the areas in the reactor system in which a critical value is exceeded become too large. But again, these critical volume fractions of the cultivation vessels do not deliver the desired insights.

Another approach to evaluate the flow in the reactor and its influence on the process could be to judge the hydrodynamic heterogeneity, e.g., via variance coefficients of the hydrodynamic parameters fluid velocity, shear rate, and energy dissipation rate, as shown in [21]. It could be shown that this might be a useful parameter to estimate the suitability of a cultivation system. The calculated variance coefficients of all hydrodynamic parameters were higher in a 3-stage-setting than in a 1-stage-setting, which might explain the rather stable process behaviour in multiple impeller systems due to the improved hydrodynamic homogeneity. Multi-stage bioreactors are quite common in industrial scale, e.g. single-use reactors with a culture volume of up to 2000 L [12]. These also involve combinations of axial and radial stirrers, which ensure both sufficient mixing and a flow field that is as homogeneous as possible [22]. Nevertheless, it can be assumed that hydrodynamic heterogeneity as the sole process design parameter will also reach its limits. The parameter could provide information about whether the stable operating range of a system is wider or narrower. However, hydrodynamic heterogeneity alone cannot be used to predict possible cell damage and the associated reduced cell growth and reduced antibody productivity. Therefore, multi-parameter approaches should always be taken into account when designing the process [15]. Due to the many overlapping hydrodynamic effects that are not just limited to stirring, this seems to be a sensible approach.

4 Recommendations for Process Development

The previously discussed observations of hydrodynamics and biological process behaviour show that there are fundamental dependencies, but that these cannot be tied to one universally applicable parameter. So far, the sole use of one of the discussed parameters is not sufficient for system-independent prediction of the process behaviour of cell culture processes. Although the relationships between the hydrodynamic parameters and the biological process parameters examined seem to be largely consistent for a specific bioreactor design, they fail when transferred to another reactor design or scale. Therefore, the observations made suggest that in order to estimate the effects of the hydrodynamics on cell culture processes, several parameters and their spatial and temporal distributions in the system must be considered.

Although the shear sensitivity of cell cultures is generally taken into account in reactor design [12], the heterogeneities of the fluid seem to be given little attention. For commercially established bioreactor systems, such as those used in production processes, usually the established process design parameters, such as the average volume specific energy dissipation rate , the mixing time, the volumetric mass transfer coefficient, the stirrer tip speed and height-to-diameter ratios as well as stirrer-to-vessel diameter ratios are given and compared [32]. However, local maxima and in homogeneities are usually only considered qualitatively in the form of visualizations of CFD simulations. In the last few decades, CFD has increasingly become an indispensable tool in process design [13–15]. With the help of CFD simulations, hydrodynamic variables in bioreactors can not only be averaged for the entire system but also calculated locally. As computers become more and more powerful, it is becoming increasingly easier to simulate complex processes even with larger gradients [33].

Due to the unclear relationships between hydrodynamic parameters and the behaviour of cells in culture systems, process development still relays on respective experiments, e.g., scale-down-models [9, 15]. Since cultivation experiments cannot be dispensed with purely predictive approaches, semi-predictive approaches, which provide for a targeted, reduced selection of experiments, should be pursued. The knowledge gained in this way can then lead to a deeper understanding of the process and can be used to formulate a strategy for process design with regard to hydrodynamics in cultivation systems for cell culture processes.

The strategy used to characterize the influence of locally resolved hydrodynamic parameters on mammalian cell cultures can in principle also be used for process development (compare [21]). If new reactors and/or cell lines are to be used for cell culture processes, it is recommended to first hydrodynamically characterize the reactor system using CFD methods for all simulated stirring, shaking or tilting speeds. Not only average values for quantities such as the energy dissipation rate, but also local maxima and distributions should be taken into account and checked for anomalies. These abnormalities can be high local energy dissipation rates, shear rates or similar. Even larger areas in which the hydrodynamic parameters reach high values can be an indicator for hydrodynamic-related problems in the process. It is therefore recommended to compare the frequency distribution of the energy dissipation rate in the reactor with the distribution of the critical energy dissipation rate. The latter can be created from the cell size distribution of

the cell line to be cultivated. If the two distributions hardly or not at all overlap, the corresponding stirrer speed should be suitable for cultivating the cell line. In parallel to the system characterization, initial cultivation tests can be carried out over the entire hydrodynamic operating range in order to be able to narrow down the proven acceptable range and ideal operating point based on cultivation data. It is advisable to proceed in nesting intervals in order to minimize the number of necessary cultivation experiments. In any case, the most production-relevant parameters such as cell growth, product formation and product quality should be examined. Are the desired observations, such as, for example, a possible operating optimum or a negative effect of flows, the corresponding range can be examined in more detail with repeated tests around the respective operating point and the observations can be statistically validated, such as required in today's regulatory files. In the event that the process behavior of the cell line under certain hydrodynamic conditions is already partially known, the existing knowledge can be used with more detailed examinations of the CFD data to limit a possible optimal operating range from the outset. This and a combination with design-of-experiments (DoE) methods can reduce the experimental effort and the duration of process design [34].

5 Conclusions

The link between hydrodynamics and biological process behaviour is still not fully understood, especially with respect to process design. Common methods to describe dependencies refer mostly to averaged hydrodynamic parameters obtained for individual cultivation systems. Hence, the use of conventional, mostly averaged process design parameters, needs to be questioned and rather, local gradients should be considered, especially for scale-up [22, 29]. Furthermore, it was found that the Kolmogorov length scale hypothesis might not be appropriate to describe the influence of hydrodynamics on mammalian cell culture processes without a hydrodynamic characterization of the full cultivation system including all hydrodynamic gradients. Overall, a deeper insight into local hydrodynamic gradients in cell culture reactors is needed, as the reliable prediction of design parameters prior to an experimental evaluation still remains difficult.

Despite the importance of hydrodynamic effects for design of cell culture processes, the knowledge on appropriate operation ranges is limited. Most published studies have been undertaken in small scale bioreactors. It is obvious, that different effects occur on a large reactor scale than on a small one. To some extend this has been addressed in scale-down-studies [9]. Nevertheless, more studies linking hydrodynamic and cellular effects especially on large scale are required.

In addition to hydrodynamic effects induced by the impeller, this also applies to bubble-induced effects, which has not been considered here. In the older literature, the significance of bubble-induced effects is considered to be less significant [11, 17]. Nevertheless, new publications indicate that different effects occur on a large reactor scale than on a small one. These can be attributed, for example, to the surface tension of gas bubbles, which cannot be changed during scale transfer. It was observed that small-scale gas bubbles introduced into a stirred tank are broken up by the stirrer and reduce the power input, while the bubbles in the large stirrer are carried to the wall of the reactor by the wake vortex generated [23]. Effects like this could provide a possible explanation for the different cell growth in different reactor designs.

In conclusion, with respect to design of biopharmaceutical production processes hydrodynamic effects on the mostly well adapted and robust cells does not seem to be that critical anymore as it used to be, but still has to be considered for optimization or process parameters. An intelligent interlinking of experimental and model-based (CFD) approaches can lead to an increased process understanding.

Conflict of interest statement

The authors declare no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Ralf Pörtner is senior research associate and group leader at Technical University Hamburg, Institute of Bioprocess and Biosystems Engineering and Honorary Professor at TH Mittelhessen University of Applied Sciences, Giessen. His main research interests are development and optimization of bioreactor techniques and process strategies, in particular for cell cultures and microbial reactions, including model-based control concepts, and tissue engineering.

Fabian Freiberger studied Bioprocess Engineering at Hamburg University of Technology (TUHH), Germany. He afterwards worked as research associate at the Institute of Bioprocess and Biosystems Engineering, where he also did his Ph.D. His research focused on model-assisted process development approaches, mainly the investigation of hydrodynamic effects in mammalian cell culture processes. Currently, he is employed in Research and Development in food ingredients and bionutrients manufacturing.

Johannes Möller studied Bioprocess Engineering at the Hamburg University of Technology (TUHH), Germany, where he did his Ph.D. and worked as a scientist at the Institute of Bioprocess and Biosystems Engineering. His research focused on accelerated and knowledge-driven bioprocess design and optimization using novel statistical and computational methods. Currently, he works in Research and Development in the pharmaceutical industry. Additionally, he is employed as an external scientist and lecturer at TUHH.

Symbols used

EDR	[W m^{-3}]	specific energy dissipation rate
$vEDR$	[W m^{-3}]	volume specific energy dissipation rate
$vEDR_{av}$	[W m^{-3}]	average volume specific energy dissipation rate for STR's, equal to P/V
$vEDR_{SP}$	[W m^{-3}]	volume specific energy dissipation rate determined from averaged slize plots
$vEDR_{SP,max}$	[W m^{-3}]	maximal volume specific energy dissipation rate determined from averaged slize plots
P	[W]	power input
P/V	[W m^{-3}]	(average) volume specific power input
V	[m^3]	volume
VCD	[cells mL^{-1}]	viable cell density

Greek symbols

μ	[h^{-1}]	specific growth rate
μ_{max}	[h^{-1}]	maximum specific growth rate

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