Overcome the Fear of S

HEAR

When mixing biologicals, concerns about shear sensitivity can be exaggerated. Careful scaledown, and the use of reference equations and CFD can help ensure success.

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The wrong choice of mixing equipment can damage or destroy shear-sensitive biological products, so optimizing mixing operations for these materials is essential. However, concerns about shear sensitivity can often be overblown, and fear can impede rational process development.

When working with any biological material, the key questions to ask are whether that material really is shear sensitive, what maximum shear it can tolerate, and what options are available to meet those criteria. For a material that is extremely shear-sensitive, diffusion may be the only safe way to achieve mixing. However, most biologicals are more tolerant of shear than one might think.

Scaledown is an extremely important part of mixing process development. At the outset, it is important to realize that laboratory mixing equipment can operate at shear rates much higher than those of industrial-scale mixers and to consider that during process development.

This article will review the concepts of shear as they relate to biomaterials (Box), and offer some guidance on optimizing mixing operations for biologicals.

Although the subject of shear may seem straightforward, surprisingly little has been written on the subject of shear and mixing [1,2], and only one reference provides actual turbulent-flow measurements for the three basic types of impellers [3].

Most people assume that the impeller tip speed (TS=BND) is most responsible for the maximum shear rate, γ_{max}, but this is only true for radial flow turbines such as Rushton turbines and paddles (Equation 1). The maximum shear rate of true axial flow impellers such as hydrofoils is only a function of impeller speed, N (Equation 2). For mixed-flow impellers such as pitched bladed turbines, both factors come into play (Equation 3).

1) $\gamma_{max,\ radial} = K_{max} \cdot N \cdot D^{1}$

2) $\gamma_{max,\ axial} = K_{max} \cdot N \cdot D^{0}$

3) $\gamma_{max,\ mixed} = K_{max} \cdot N \cdot D^{0.5}$

The significance of the impeller diameter, D, depends upon the flow pattern created by the impeller. K_{max} is a function of the impeller type. Interestingly, the average shear rate, $\gamma_{average}$, shows the same dependency regardless of flow pattern (Equation 4).

4) $\gamma_{average} = K_{avg} \cdot N$

In general, high-speed radial flow turbines generate the highest shear rates, followed by the mixed-flow impellers and the slow-speed true axial-flow impellers. Table 1 on p. 28 shows K-factors measured in the discharge zone of the impeller, a slight distance from the edge of its blade.

SCALEUP CONSIDERATIONS

These equations have some interesting implications for process scaleup. In all cases, as impeller speed, N, increases, so do the average and maximum shear rates. Therefore, except for true axial-flow impellers, an increase in the impeller diameter increases the maximum shear rate at the same impeller speed. If shear sensitivity is a concern, radial flow impellers should not be used. In the case of hydrofoils, scaleup will always result in a lower shear rate. All small-scale mixers operate at high impeller speeds: the typical bench top mixer may run at over 1,000 rpm, while large industrial-scale mixers may run as low as 30 rpm. In this case, the average large-scale impeller shear rates of all impeller styles will be only 3% that of the small-scale mixer. The maximum shear rate difference depends on the impeller diameter and the

MIXING 101: A BASIC REVIEW

As a mixer's impeller rotates, it creates flow. Depending on the type of impeller involved, the flow patterns resulting can be very different and a single point in a mixing tank will experience a wide range of fluid velocities, turbulence and shear rates during the mixing process.

Shear is a velocity gradient, measured in 1 per time or s^{-1}, and defined as the ratio of the difference between the fluid velocities of two close points in a tank over the distance between these points.

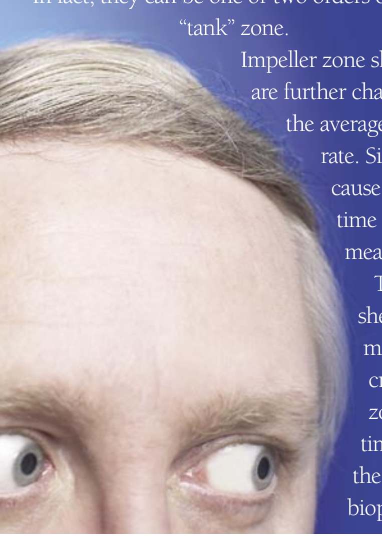
$$\gamma = dU/dy$$

Any bioreactor equipped with a mixer will experience a wide variety of shear rates in different regions. The highest shear rates will be near the impeller blades, in the "impeller zone." Shear rates around baffles or other obstacles can also be high.

Shear rates are lower the farther away they are from the impeller blades. In fact, they can be one or two orders of magnitude lower in the "bulk" or "tank" zone.

Impeller zone shear rates are most critical, so they are further characterized by measurements such as the average and the maximum impeller shear rate. Since fluctuations in fluid velocity can cause shear rates to vary, they are typically time averaged when they are being measured.

The average and maximum impeller shear rates are the average and maximum of all of the shear rates created by the impeller in its discharge zone. Depending on the residence time and the flow rate generated by the impeller, either one can determine a bioproduct's shear sensitivity.



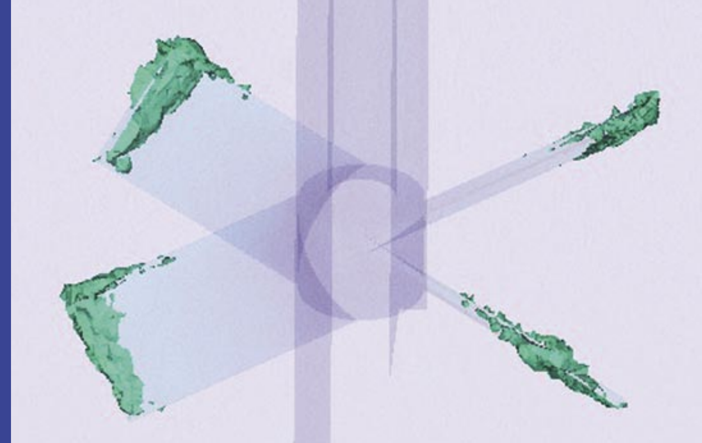


Figure 1: Region of impeller shear rates greater than 4000 s^{-1} for a pitched bladed turbine (PBT), D = 203 mm, T = 610 mm, N = 120 RPM, ρ=1000 kg/m^3, η=1000 cPs.

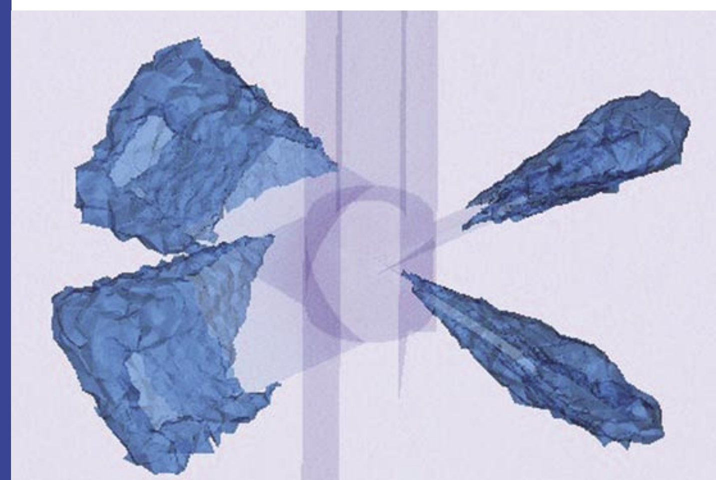


Figure 2: Region of impeller shear rates greater than 1000 s^{-1} for a pitched bladed turbine (PBT), D = 203 mm, T = 610 mm, N = 120 RPM, ρ=1000 kg/m^3, η=1000 cPs

impeller flow pattern. If we assume the small-scale impeller diameter, D, is 50 mm, and the full-scale D is 2500 mm, the full-scale maximum impeller shear rate will be 150%, 21%, or 3% of the small-scale radial-flow, mixed-flow, or axial-flow impeller, respectively.

To illustrate the effect of impeller geometry and scaleup on shear, let us assume that power per unit volume, P/V, is to be kept constant and equal to 0.4 kW/m^3. We will compare a Rushton turbine, a 45° pitched-blade turbine, and a hydrofoil, such as the A310 (photo, p. 31). Assume that the tanks have flat bottoms with a liquid level equal to the tank diameter and the density of the broth equaling 1100 kg/m^3. The small-scale tanks will have 21.2 L and T equal to 300 mm. The large-scale tanks will have 21,200 L and T of 3,000 mm. The linear scaleup ratio is 10:1, whereas the volumetric scaleup ratio is 1000:1.

Impeller power, P, which is the power dissipated into the reactor, is calculated by using Equation 5, where Np is the dimensionless power number.

$$5)\ P = \rho_{fluid}\, Np \cdot N^3 \cdot D^5$$

The flow rate, Q, generated by the impeller is given by Equation 6, where Nq is the dimensionless flow number.

$$6)\ Q = Nq \cdot N \cdot D^3$$

Recirculation time is the time it takes for the average particle to return to the point it started at. If we assume that it requires three recirculations to mix the tank [4], the mixing time, t_{mix}, is given by Equation 7. Please note that this relation is somewhat oversimplified, but makes a convenient rule of thumb for turbulent flows.

$$7)\ t_{mix} = 3 \cdot \frac{V}{Q}$$

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TABLE 1: CHARACTERISTIC NUMBERS OF THREE BASIC IMPELLERS

	Flow Pattern	K_{avg}	K_{max}	Np	Nq
Rushton turbine	Radial	12	145.7/m	5.75	0.80
45° pitched bladed turbine	Mixed	5.4	33.9/$m^{0.5}$	1.27	0.79
A310 Lightnin hydrofoil	Axial	3.4	6.6	0.32	0.56

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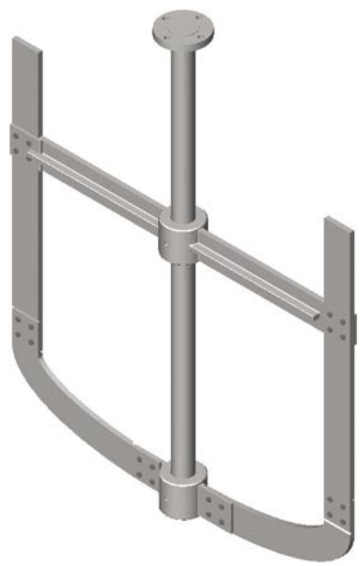
Table 2 compares the three basic impeller types with respect to mixing and shear rates. It is obvious that the hydrofoil requires the greatest tip speed to achieve the required P/V, but it has much lower average and maximum shear rates than the pitched bladed turbine (PBT) and Rushton turbine. The difference may not be all that apparent at small-scale, but becomes obvious in large-scale installations. Where the maximum shear rate increased 113% for the Rushton turbines, the maximum shear rate decreased 32% for the PBT and decreased 78% for the hydrofoil. Since the impeller speed is always less on scaleup at constant P/V, the average shear rates are always less, regardless of impeller types. This means that the shear rate spectrum within the tank changes dramatically upon scaleup. The mixing times are much shorter using the hydrofoils, too.

Assuming that tests revealed that reactants showed a shear rate sensitivity of over 20 s^{-1}, none of the small-scale mixing experiments would have indicated a positive result. Yet, all three impeller types might work at the industrial scale, depending upon which shear rate is responsible for shear damage. Just because lab experiments indicate shear sensitivity does not mean that the same problems will be seen at scaleup.

To determine which impeller shear rate is determining the process, one must calculate the mixing time (Equation 7) and compare it to the batch time or residence time (for continuous processes). A batch time that is much longer than the mixing time indicates that the average particle or species travels through the impeller zone more frequently, and will eventually travel through the zone of maximum shear rate. In such cases, the maximum impeller shear rate will be responsible for the process.

If the species does not, on average, travel through the high shear zone, then the average impeller shear rate dictates the process. On the small scale, maximum impeller shear rate is usually responsible because of the very short mixing times involved. On larger scale operations, mixing times are always longer, and if the fluid involved is viscous, the mixing times may be much longer than Equation 7 suggests, so the average impeller shear rate may be more important.



An anchor impeller assembly.

DETERMINING THRESHOLD SHEAR RATE

In all too many process development efforts, the threshold shear rate is determined by accident. The experiment is set up in a small lab-scale mixer (or even using a magnetic stirrer). After running tests and noting a failure, microscopic examination of the biomaterial indicates that cells in the bioreactor have been damaged.

This is a good time to think about scaledown. Some questions to ask would include:

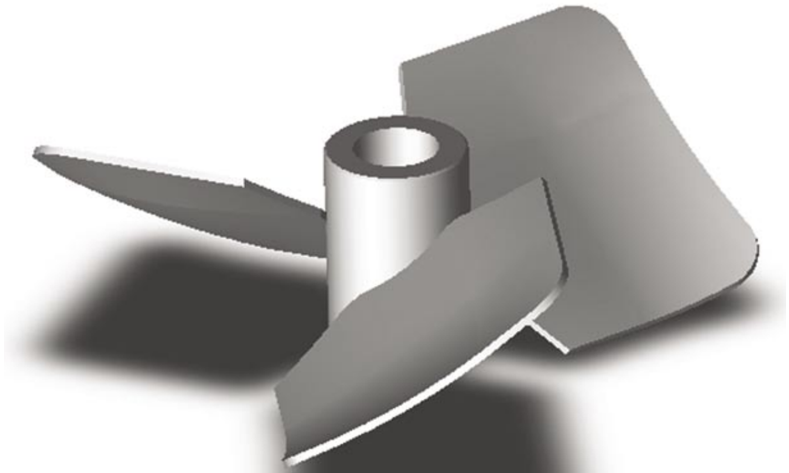
- What would the full-scale reactor look like if the reaction succeeds?
- What commercial reactors are currently available?
- Have reactors been running similar processes with any success? If so, what are their impeller shear rates?
- Can we reproduce the characteristics of those commercial reactors in the lab?

Data from Table 1 can serve as a good starting point, and then the reactor can be scaled down.

Methodical experimentation is needed to determine the actual shear rate sensitivity of the bioprocess reactants. If, at one speed, shear damage is noticed, the impeller speed should be reduced until either damage is no longer seen or is within acceptable limits.

TABLE 2: COMPARISON OF THREE IMPELLER DESIGNS: ρ_{fluid}=1100 kg/m^3, P/V=0.4 kW/m^3

	T	D	N	TS	P	γ_{avg}	γ_{max}	Q	t_{mix}
units	mm	mm	rpm	m/s	Watt	1/s	1/s	L/s	s
Rushton turbine	300	100	307	1.6	8.5	61	75	4.09	15
45° Pitched bladed turbine	300	100	508	2.7	8.5	46	91	6.69	9.5
A310 Lightnin hydrofoil	300	120	594	3.7	8.5	34	65	9.6	6.6
units	mm	mm	rpm	m/s	kW	1/s	1/s	L/s	s
Rushton turbine	3000	1000	66	3.5	8.5	13	160	880	72
45° Pitched bladed turbine	3000	1000	109	5.7	8.5	9.8	62	1435	44
A310 Lightnin hydrofoil	3000	1200	128	8.0	8.5	7.3	14	2064	31



An A310 hydrofoil turbine.

If no damage is seen at the first speed tested, increase the speed until shear damage is noticed. Equations 1-4 should provide a clear idea of what the threshold shear rate is. For expensive compounds, generally, start with the lowest speed and increase it gradually.

PREDICTING SHEAR DAMAGE

Kolmogorov's theory is beyond the scope of this article, but since it is often used to predict shear damage, we'll touch on it briefly (for more information, see Reference 2, Chapter 18). According to this theory, if isotropic turbulence exists (and it most likely does not in a bioreactor), then the energy from the impeller will dissipate down to a microscale of turbulence, λ_K, which is only a function of the kinematic viscosity, ν, of the broth and the local energy dissipation, ε_T, as described in Equation 8. ε_T is the power per unit mass, or $P/\rho V$.

$$8)\ \lambda_K = \left(\frac{\upsilon^3}{\varepsilon_T}\right)^{(1/4)}$$

If we assume waterlike fluids and $P/V = 1\ kW/m^3$, then the value of λ_K is 30 µm. Since *E. coli* cells are 1 to 2 µm, they should not be affected by the turbulence generated by the impeller. However, fungal and filament fermentations, which often involve materials with longer branches, could be damaged. This is not a theory based on shear, but on the intensity of turbulence, which is directly related to the magnitude of the fluctuating velocity factor. It is like a time-dependent shear rate. Equation 8 would be useful in providing a first estimate.

MODELING, USING CFD

Using commercial computational fluid dynamics (CFD) software such as Acusolve by Acusim (Mountain View, Calif.), can provide insights into the mixing process by considering the appropriate combination of the "velocity gradient tensor" (normally we calculate the "strain rate magnitude") [5].

Graphical output is shown in Figures 1 and 2 (p. 28) for laminar flow ($Re = 82 = \rho ND^2/\eta$). Shear rate calculations generated by CFD programs are much more precise than correlations found using Equations 1-4. In addition, the software can show areas of concern graphically. Obviously, when the flow is not turbulent, the shear rates become much greater than Equations 1-4 will predict.

If reactants are extremely shear sensitive, lower impeller speeds will reduce the shear rate. To get a mixing action, though, the impeller diameter required would be quite large, and there are some limitations. Hydrofoils should not be larger than 70% of the tank diameter, because if they are any larger, they will generate very high shear at the impeller tips. In general, radial turbines or pitched bladed turbines should not be used with very shear-sensitive materials. If either of

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these types must be used (for other mixing requirements), they should be used with baffles.

Large, slow diameter impellers include the anchor, helical ribbons, the Paravisc from Ekato (Schopfheim, Germany; www.ekato.com), and the glass-lined Retreat Curve impellers manufactured by Pfaudler (Rochester, N.Y.; www.pfaudler.com). The anchor, helical ribbons, and the Paravisc have close tank wall clearances and are usually used in high viscosity applications without baffles, but can also be used in low-viscosity fluids for this purpose.

The anchor impeller (photo, p. 30) creates essentially a tangential flow pattern, which is conducive for very low shear and poor mixing. Use Equation 2 and K = 25 to predict the shear rate [2] of anchors. Helical ribbons and the Paravisc add an axial component to the tangential flow pattern, which does improve the mixing, but increases the shear slightly. Use Equation 2 and K = 30 to predict the shear rate [2] of helical ribbons.

Glass-lined Pfaudler Retreat Curve impellers also create a tangential flow pattern and, thus, low shear rates. These impellers are placed close to the bottom of the tank and their diameters may range from 70-95% that of the tank. Since the impellers and baffles would be glass-lined and round, they offer a low shear and low impact environment. The K-factors of the Pfaudler impeller are not published, but should be about the same as those for the helical ribbon.

There is another interesting impeller worth mentioning, which has not yet been used in biochemical reactors. The Spirok impeller patented by the Finnish mining company, Outokumpu Technology (Espoo, Finland) and used in the extraction of copper, has all the characteristics desired from a low-shear impeller [6]. Used to disperse two immiscible phases with very little shear, it resembles a round-bladed helical ribbon with support cross-arms covering the entire height of the reactor and is designed for 70% of the baffled tank diameter. This is the diameter that dissects a tank volume into two equal parts. Due to its round blades, this impeller could be easily cleaned in place and damage due to impact on the blades would be minimized.

If air needs to be dispersed into a process involving shear-sensitive media, up-pumping is the solution. Radial impellers, which are most often used to

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disperse gasses, impart too much shear. Axial down-pumping impellers usually have to run at such low speeds that they cannot effectively disperse the air.

Up-pumping impellers require large diameter ratios (55-65% of the tank diameter) in order to function correctly, so the impeller speeds will be lower. Generally, an additional up-pumping impeller will be needed to direct the flow. Wide-blade impellers are also better to use because they have a higher power number, Np, which further decreases the impeller speed to achieve a certain power input. Up-pumpers have been successfully used with animal and plant cells.

In short, although shear sensitivity is a major, and justifiable, concern in biopharmaceutical manufacturing, it is often exaggerated. Without shear, there can't be any mixing, and if any biomaterial is so sensitive that it can't tolerate shear, then it should be mixed via diffusion.

Biopharmaceutical manufacturers shouldn't fear shear, but exploit it effectively and verify it empirically, determining shear threshold with well-defined impellers rather than magnetic stirrers or glass lab equipment. Known relations, summarized in equations 1-4, and techniques like CFD can add more knowledge, and decrease fear, improving mixing process scaleup.

About the Author

Dr. Tom Post has worked as an independent consultant in fluid mixing for the past five years. Before establishing his own business, he was VP of mixing technology and R&D at Lightnin, and held a number of other key positions at that company; he also worked in the pharmaceutical industry for Smith Kline and French. He has coauthored one book, and is the author of four patents and numerous articles on mixing. He is also knowledgeable about separations technologies, including chromatography. Dr. Post has a Ph.D. from the Swiss Federal Institute of Technology in Zurich. He is PharmaManufacturing.com's resident mixing expert.

References

1. *Oldshue, James Y., "Fluid Mixing Technology," Chemical Engineering, McGraw-Hill, New York (1983) Chapters 1-2.*
2. *Paul, Edward L., et. al, Handbook of Industrial Mixing, North American Mixing Forum, Wiley-Interscience, Hoboken, NJ (2004), pp. 368-371 and chap. 18.*
3. *Weetman, Ronald J. et al, "Power, Flow and Shear Characteristics of Mixing Impellers", 6th European Conference on Mixing, Pavia, Italy, Organized by AIDIC (1988), pp. 43-50.*
4. *See www.postmixing.com for more information.*
5. *Personal communication with CFD specialist, Keith Johnson, kedmundj@neo.rr.com*
6. *http://www.outokumputechnology.com.*