25 mm vs. 50 mm orbital shaking in shake flasks

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Oxygen transfer and hydromechanical stress are key design parameters for shaken bio-processes. On orbital shakers, both are strongly influenced by shaking frequency, filling volume and vessel geometry. But what about the shaking diameter? In practice, users often have to decide between 25 mm and 50 mm orbits. This AppNote summarizes how shaking diameter affects maximum oxygen transfer capacity (OTR_{max}) and mechanical stress in shake flasks and explains why the "better" orbit depends on the application rather than on a single performance metric.

The concept of OTR_{max} and its dependence on shaking parameters has been described in detail in our AppNote Maximum oxygen transfer capacity and the underlying correlation of Meier et al. (3) for shake flasks.

Oxygen transfer and the role of shaking diameter

In orbitally shaken flasks, oxygen transfer is driven by rotation of the bulk liquid and formation of a thin liquid film on the flask wall while continuously renewing the gas–liquid interface.

Larger shaking diameters increase the centrifugal force, acting on the liquid. At otherwise identical conditions (same flask, filling volume and shaking frequency), this leads to a larger gas-liquid interface surface area, and this leads to higher OTR_{max} values.

In real incubator shakers, the maximum usable shaking frequency is usually higher at 25mm than at 50mm orbit (due to mechanical limits and loading). This means the practically relevant question is not *"25 or 50 mm at the same rpm?"* but rather *"25 mm at higher rpm versus 50 mm at lower rpm?"*

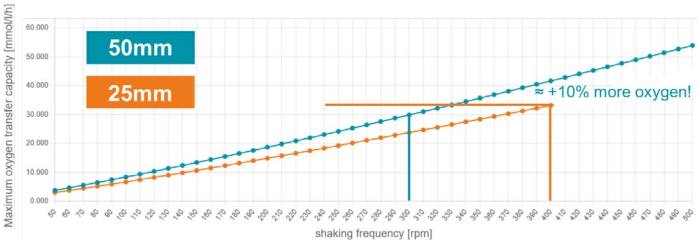

Figure 1: Calculated OTR_{max} values (4) according to Meier et al. (3)

In figure 1, the 50mm curve is above the 25mm curve at equal shaking frequencies.

However, there are different shaking frequency limits in place (300 rpm at 50mm and 400rpm at 25mm). The OTR_{max} achieved at 25mm/ 400rpm is slightly higher – by roughly 10 % – than at 50mm/ 300rpm. The underlying reasons are described in detail by Schulte at al. (2).

In conclusion: If the shaker can be operated at substantially higher shaking frequency with the smaller 25 mm orbit (e.g. 400rpm), this higher frequency overcompensates the smaller orbit and delivers higher overall OTR_{max} values than 50 mm at its limit of e.g. 300rpm (2),(3).

Mechanical stress and power input

Mechanical stress in shake flasks arises mainly from velocity gradients in the liquid, especially near the flask wall, where laminar shear dominates. Flow field visualizations show that the highest liquid velocity changes – and thus shear stresses – occur in the thin liquid film running around the wall during orbital motion (7).

Often, the following metrics are used to quantify different levels of hydromechanical stress (5),(6):

- specific power input (P/V, $W \cdot m^{-3}$),
- energy dissipation rate (ε, $W \cdot kg^{-1}$)
- maximum local energy dissipation rate (ε_{max}, $W \cdot kg^{-1}$)

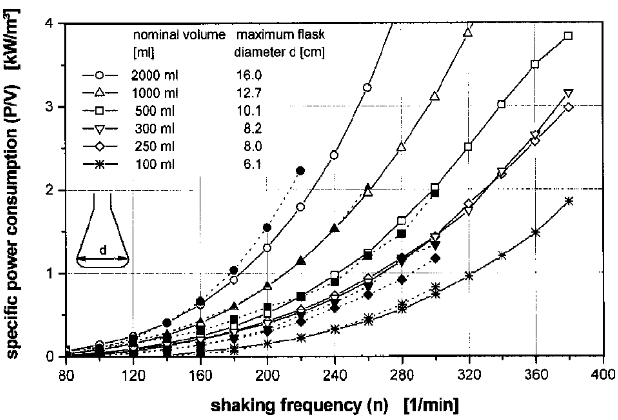

Figure 2: Power consumption in shake flasks, each filled with 1/10th of the nominal flask volume, solid lines 25mm shaking diameter, dotted lines 50mm shaking diameter, from Büchs et al. (5)

For unbaffled shake flasks at low viscosities, at laminar flow conditions (Reynolds number < 60.000) experiments show that these stress-related quantities depend strongly on shaking frequency and flask diameter,

while the shaking diameter enters only weakly or not at all into the correlation (1),(5),(6).

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Putting it all together: Application-driven choice of orbit

Because oxygen transfer and mechanical stress respond differently to shaking diameter and frequency, the "best" orbit depends on the biological system and the available operating window.

Stress-resistant and fast-growing organisms

When the shaker is used at high shaking frequencies that are only feasible at 25 mm orbit (above ~360 rpm), the higher liquid velocity leads to higher OTR_{max} values than 50 mm at its lower maximum frequency. In the example comparison, this results in about 10 % more oxygen transfer for 25 mm at full speed compared to 50 mm at its maximum frequency.

This is particularly attractive for oxygen-demanding, shear-tolerant microbial processes (e.g. *E. coli*, yeasts) or high-rate production or screening experiments where OTR is the main limitation.

Stress-sensitive and slow growing organisms

When the process is not operated at high shaking frequencies accessible with 25 mm (e.g. below ~360 rpm in the discussed example), the larger 50mm orbit can achieve the same OTR_{max} as a 25 mm orbit while operating at a lower shaking frequency.

Lower shaking frequencies lead to reduced power input/ energy dissipation rates. This is advantageous for shear-sensitive cell culture applications (e.g. mammalian suspension cells) where excessive shear or energy dissipation should be avoided.

Practical recommendations:

1. Define the primary limitation first: Oxygen supply vs. mechanical stress.

2. Use 50 mm orbit when:
- operating below the maximum rpm of the shaker
- working with shear-sensitive cell culture systems and targeting minimized hydromechanical stress.

3. Use 25 mm orbit when:
- exploiting the higher maximum shaking frequency of the shaker
- running oxygen-demanding (microbial) processes, and mechanical stress is not the primary concern.

4. Whenever possible, keep both options available.

Projects, organisms and process requirements change. Having both 25 mm and 50 mm orbits on the same shaker platform allows users to adapt shaking conditions to the specific needs of each application, rather than compromising on a "one-size-fits-all" orbit.

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