Optimization of mixing parameters in lab automation for high & low sample volumes

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Abstract

In the past the significance of microplate mixing has mainly been ignored as a serious problem, although potentially it could undermine a generation of meaningful data.

We analyzed orbital microplate mixing technologies to get a better understanding of the current requirements for mixing technologies that can be applied to compounds or bioassays in microplates.

This poster reviews some results of studies with BioShake orbital plate shaker, such as where the greatest need for improved mixing of high sample volume in standard tubes against low sample volume in HTS microplates.

It also discusses the influences of construction material, shape and well volume of the sample carrier to the mixing results depending on the physical properties of the liquids.

Introduction and Background

Orbital shaking is undoubtedly a simple and non invasive way for mixing of assay components. Simply putting the samples on a shaker table doesn't guarantee that a complete blending is reached after the mixing process.

It is important to choose the most appropriate process parameters in dependency of the sample volume and the geometry of the sample container.

The following section describes the mathematical background of calculating the minimal necessary mixing frequency for exceeding the surface tension of the medium, depending on the filling volume, the shaking diameter and other physical and geometrical parameters.

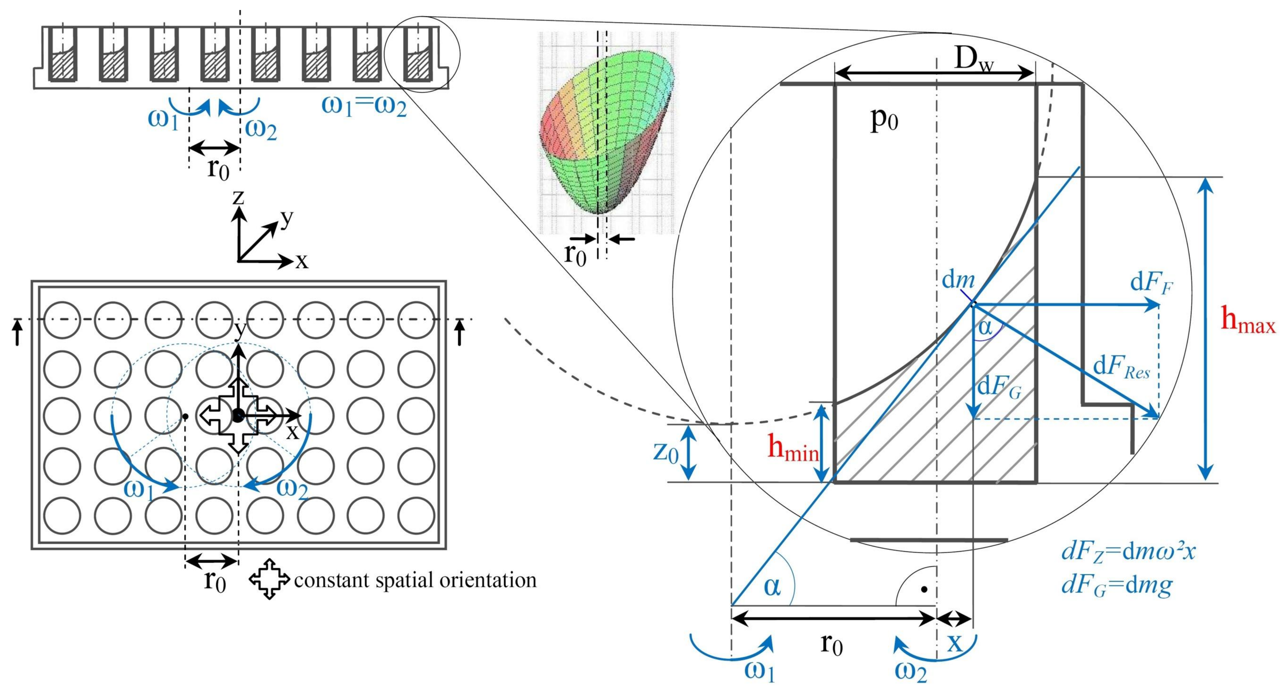

Figure 1: Orbital movement and resulting fluid distribution in a microplate well.

In Figure 1 the resulting liquid distribution in a well due to the orbital movement of a microplate is shown. If the effect of friction and surface forces is neglected the free surface of the fluid, which is a face of an equal pressure level, forms a rotational paraboloid.

The acting forces to a volume element d_m within the free surface are gravity and centrifugal force. In a co-moving reference frame the fluid appears to rotate along a stationary wall. Interesting values are the minimal and the maximal height of fluid depended of amplitude d_0 and mixing frequency n. Further Information about calculating liquid distribution can be found in [2].

The choice of appropriate operating parameters for orbital mixing, especially the mixing frequency n and the amplitude d_0, is depending on:

- microplate filling volume V_F,
- well geometry (diameter D_W, height h),
- surface tension of fluid and construction material σ,
- fluid density ρ,
- and kinematic viscosity of the fluid ν.

The most important requirement for an effective mixing process is the formation of a macroscopic flow. As microplate well volumes decrease the impact of surface tension increases because of the low volume/surface ratio of the usually thin and tall well geometry. For this reason it is necessary to generate a high centrifugal acceleration to achieve an intensive macroscopic flow. A large number of commercially available instruments have been developed for use with larger laboratory vessels and they are not designed to generate a centrifugal acceleration which is required for processing small volumes. The labour required for surface enlargement must be delivered by the centrifugal force. The increased centrifugal force exceeds the surface tension at a critical shaking frequency [3]

$$n_{\min} = \sqrt{\frac{\sigma D_W}{4\pi V_F \rho d_0}},$$

with the surface tension σ, the well diameter D_W, the fill volume V_F, the fluid density ρ and the amplitude d_0.
The value of centrifugal force respectively acceleration depends on the amplitude d_0 and the mixing frequency n. Against expectation it is crucial to choose the right value of amplitude d_0.

Test Methods

The minimal necessary value of mixing frequencies has been calculated using the formula shown in the previous section.
All sample containers have been mixed using a BioShake orbital plate shaker to find the optimal mixing frequency.

The basic requirement to ensure a fast and efficient mixing result is the generation of a vortex which allows to produces a flow of the total sample volume. For observing the movement of the fluid in the samples container during the process at different mixing frequencies a high speed camera system (The Imaging Source®, DFK 21BU04) was used. The knowledge obtained was used for optimization of mixing speed and amplitude. The results of the calculated minimum necessary mixing frequency have been compared to the experimentally determined frequency which leads to a movement of the fluid in the sample containers.

Results and Discussion

Figure 2 shows the calculated start of mixing effects in dependence of the orbit, shaking speed, filling volume and geometry of the sample container.

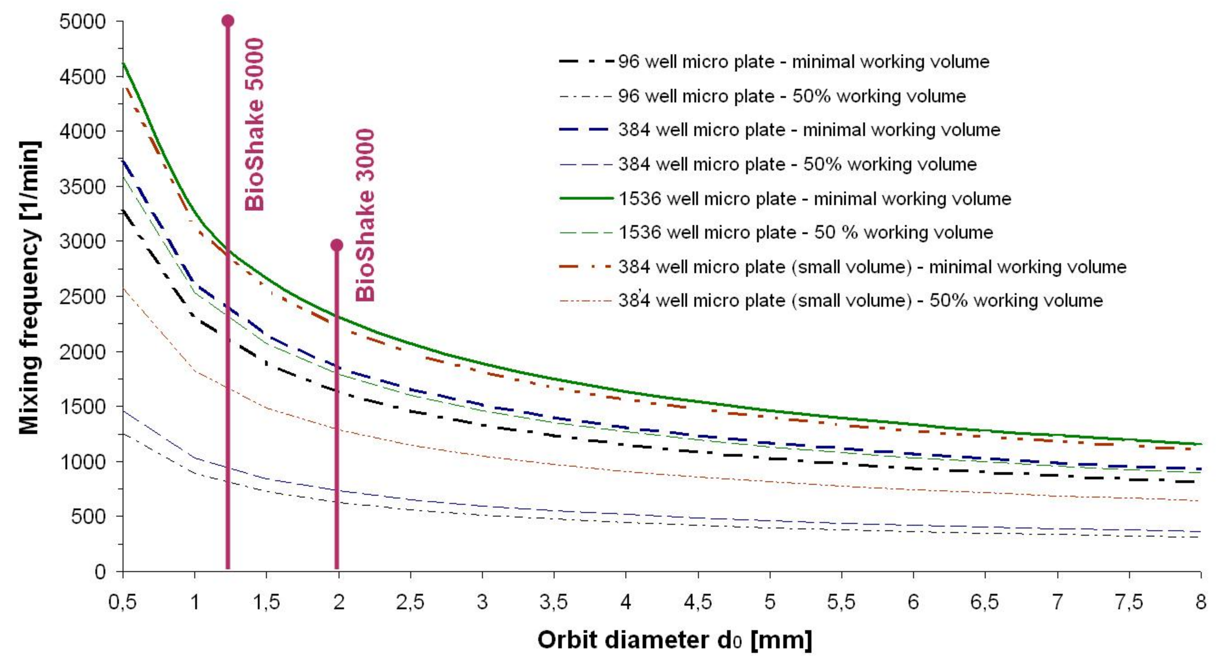

Figure 2: Minimal frequencies [rpm] for starting mixing effects for different microplates depending on orbital diameter and filling volume/well [%] for diluted fluids at 20°C

Figure 3: Experimental studies of the mixing behavior in a 96 well plate depend on preselected mixing speeds. For aqueous solutions with 210 µl filling volume/well the ideal shaking frequency should amount 1,200 rpm. Below 1,000 rpm, only inadequate mixing results can be reached in a short time. From 1,400 rpm start of the danger zone of possible spilling effects of the samples.

The adjustment of the optimal mixing frequency for microplates or tubes should always be made in dependence on the size of the well or tube and the filling volume. Only in this way optimum results can be achieved with in shortest process time with highest reproducibility.

The calculated data in Figure 2 fit very well to the measured data.

Mixing speeds [rpm] for different microplates depending on filling volume/well [%] *				
Filling volume in %	96 well *(standard)*	384 well *(standard)*	384 well *(small-vol.)*	1536 well *(standard)*
10 %	1800 - 2200	2200 - 2600	2800 - 3000	4000 - 5000
25 %	1600 - 2000	2000 - 2400	2400 - 3000	3500 - 4500
50 %	1400 - 1800	1800 - 2200	2200 - 2600	2800 - 3500
75 %	1200 - 1600	1600 - 2000	2000 - 2400	2500 - 3000

Table 1: Shows an short summary for recommended mixing speeds for microplates. All values refer to diluted fluids and mixing orbit of 2.0 mm. Values for 1536 well plates refer to mixing orbit of 1.2 mm.

Mixing speeds [rpm] for tubes/vials depending on filling volume [%] *				
Filling volume in %	0.2 ml tube	0.5 ml tube	1.5 ml tube	2.0 ml tube
10-50 %	1400 - 1800	1200 - 1600	1000 - 1300	1000 - 1300
50-75 %	1200 - 1500	1100 - 1300	1000 - 1200	900 - 1200
75-100 %	1000 - 1300	1000 - 1200	900 - 1100	900 - 1100

Table 2: Shows an short summary for recommended mixing speeds for tubes. All values refer to diluted fluids and mixing orbit of 2.0 mm.

Conclusion

The significance of microplate mixing is taking on increasing importance, partly in order to be able to improve bioassays in microplates. If efforts to reduce assay result uncertainty are to be truly comprehensive, then it is absolutely clear that the microplate mixing protocol needs to be included as one of the key variables to be optimized during method development.

It is very important to use appropriate parameters to mix samples in biotechnology. Larger volume should be mixed with a higher orbit and less speed. Smaller volumes require a lower orbit, but a much higher speed. For example a 96 well microplate requires at least 1200 to 2200 rpm on an orbit of 2.0 mm and a 1536 well microplate requires at least 2500 to 5000 rpm on an orbit of 1.2 mm to see mixing effects.

Mixing on the right frequency saves time and money.

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