How to calculate the minimum shaking frequency for microtiter plates

AppNote by Kuhner shaker

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Many shaken bioprocesses progress from a small scale (e.g. microtiter plate) to a relatively large scale (e.g. 2,500L bioreactors). Once a culture has been optimized for a microtiter plate (MTP), it can be scaled into larger vessels using a variety of parameters (e.g. OTRmax); however, the initial shaking conditions for a micro-titer plate are not always known. Here we discuss the key equations and relationships that can be used to determine the initial conditions for a shaken bioprocess in an MTP and illustrate an example in a 96-well plate.

Key considerations for shaking in an MTP

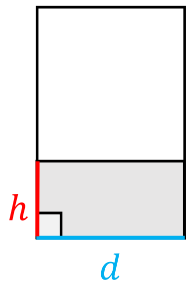
Microtiter plates (MTP) are rectangular plates with standardized dimensions (SBS) that contain a matrix of adjacent, small vessels (wells). The number of wells that can make up a plate (e.g. 96, 48, 24, etc.) will often define the size of each individual well. MTPs may vary in both the shape of the walls (e.g. circular, square, star, etc.) and the bottom surface of the well (e.g. flat, round, pyramidal, etc.). The calculations described in this note are specific for MTPs with square-shaped wells but may also be used as an approximation for other MTP formats.

When shaking MTP cultures on an orbital platform, it is important to generate enough force to ensure that the surface of the liquid contacts the bottom of the well. This ensures that the solution is fully mixed and that the cells remain in suspension. The angle formed between the surface of the liquid and the bottom plane of the vessel is referred to as the 'mixing angle' (θ). Using θ and basic trigonometry, we can relate the height from the filling volume with the centrifugal force generated from the shaking to identify the shaking frequency necessary to achieve full mixing in an MTP.

How is the mixing angle calculated?

The diagram and equations on the right illustrate how θ is defined in relation to force and filling volume. The top diagram represents an unshaken vessel, and the top equation relates the liquid height (h) to the filling volume. The lower diagram represents the same vessel when shaking with enough force to achieve a full mixing angle. The centrifugal force pointing away from the center of rotation (red arrow), the gravitational force (blue arrow), and the corresponding hypotenuse form a 90° triangle (Triangle 1) that is proportional to the 90° triangle formed by the height of the shaken liquid (h, red line), the width of the vessel (d, blue line), and the surface of the liquid (Triangle 2). Since θ is maintained in both triangles, we can calculate the 'mixing angle' for Triangle 2 by taking the tangent of the centrifugal force and the gravitational force from Triangle 1.

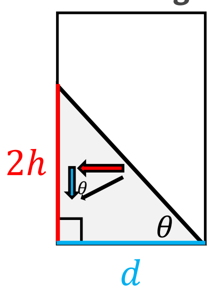
No Shaking



$$V_{liquid\ (cube)} = (d)^2 \times h$$

Adapted from Wouter Duetz, Enzyscreen

Shaking



$$\frac{Cent.Force}{G.Force} = \frac{2h}{d} = \tan\theta$$

The relationship between the triangles is illustrated here with color; the red arrow (centrifugal force) from Triangle 1 represents the same arm of the 90° triangle as $2h$ from Triangle 2. In general, when the height of the shaken liquid is twice the height of the unshaken liquid, full mixing is achieved [1-3]. The following equation can then be used to relate different shaking conditions (e.g. shaking frequency) with different filling volumes via the ratio of the centrifugal force with G-force.

$$\frac{Cent.Force}{G.Force} = 5.6 \times 10^{-7} \times \left(d_0 + \frac{d}{y}\right) \times n^2$$

The equation terms include: d_0 (shaker diameter, mm), d (vessel diameter, mm), y (diameter factor), and n (shaking frequency, RPM). The constant of 5.6 x 10^{-7} accounts for the gravitational force and unit conversions. The diameter factor (y) utilized in the above equation defines the contribution of the vessel diameter towards the overall 'effective diameter'.

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Our recommended values for this factor in the table below were empirically determined by comparing the hydrodynamics of different vessels under different shaking conditions [4-8].

Vessel	96-well (round)	96-well (square)	48-well (round)	24-well (round)	24-well (square)	50mL tube
Vessel Dia. (d)	6	8	11	16	17	30
Dia. Factor (y)	4	2	4	3	3	2

Application example: 96-well plate

The equations and relationships described above can be utilized to identify the conditions that will yield full mixing in a 96-well plate. For instance, if working in a 96-deep-square-well plate (diameter of ~8mm) with a filling volume of 0.5mL (from a total volume of 2.2mL per well), we would first calculate the filling height (h):

$$h = \frac{V_{liquid}}{(d)^2} = \frac{0.5 \ * 1000}{(8)^2} = 7.81mm$$

We would then calculate the force ratio and mixing angle:

$$\frac{Cent.Force}{G.Force} = \frac{2h}{d} = \frac{2 \times 7.81}{8} = 1.95$$

By taking the inverse tangent of the force ratio, we can also compute the mixing angle that would yield full mixing (as shown below). While the mixing angle itself is not actually needed to compute the minimum shaking frequency, it can be a useful parameter for comparing across vessels.

$$\tan^{-1}(1.95) = 63°$$

Once we have calculated the filling height (converted to millimeters here for convenience) and the force ratio, we then calculate the conditions that will provide enough force to form this mixing angle. For instance, if the shaker is set to an orbital diameter (d_0) of 50mm, the following equation would yield the minimum shaking frequency required to generate a mixing angle of 63° in these well plates:

$$n = \sqrt{\frac{1.95}{5.6 \times 10^{-7} \times \left(50 + \frac{8}{2}\right)}} \cong 254 \ rpm$$

These calculations indicate that shaking 96 square-well plates (with a ~8mm well-diameter) at 254rpm on a 50mm orbital diameter would yield full mixing with a mixing angle of 63°. These equations can be utilized for other well plates by substituting the appropriate well diameter (d), orbital diameter (d_0), and diameter factor (y).

In summary, shaken bioprocesses will typically initiate at a small scale and often in micro-titer plates (MTP). Once culture conditions are established in MTPs, they can be scaled-up using a variety of parameters (e.g. OTRmax); however, estimating these initial conditions in an MTP can be challenging. The mixing angle, which is the angle that the surface of the liquid forms with the bottom of the well (represented as θ in the above diagram), is a good indicator of mixing. In general, a mixing angle that corresponds with a doubling of the initial filling height (h) will yield good mixing and gas transfer [1].

Using trigonometry to relate the geometry of the well with the geometry of the force vectors, we can relate the centrifugal force generated by the shaker to the filling height from the liquid. Since the centrifugal force is defined by the shaking frequency and orbital diameter, we can then relate these two parameters to the filling volume and compute the shaking frequency required to achieve the desired mixing angle in an MTP.

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