APPLICATION NOTE

# Shaker Agitation Rate and Orbit Affect Growth of Cultured Bacteria

**Authors:** Mary Kay Bates;
David S. Phillips and Janet O'Bryan,
Thermo Fisher Scientific

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## Summary
Bacteria are grown in flasks on rotating shakers for many reasons, including production of non-native proteins and genetic material for research applications. The microbial growth can be affected by many variables, chief among these being temperature and aeration controlled by the mechanical orbital shaker. This article focuses on the shaking parameters and how to modify them for different shaker characteristics, including orbit diameter and rotation speed. We provide a conversion formula that allows convenient transition from one shaker with a specific orbit and speed to another shaker with a different orbit and speed.

## Introduction
Since Robert Koch formalized processes for bacterial growth and study in the 19th century, bacteria have been grown on agar plates and in liquid culture with thousands of variations. Bacteria are grown in flasks on rotating shakers for production of DNA and protein, for study of bacterial biology, or for use in food production processes.

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Growth patterns are highly sensitive to conditions including temperature, pH, oxygen concentration, nutrient concentration, vessel size, volume of the liquid growth medium, number of bacteria used for seeding cultures, and growth characteristics of the chosen bacterial species and strain. Two of these characteristics, temperature and aeration, are controlled by the orbital shaker. Since many different shakers exist that have different mechanical characteristics, it can be difficult to transition from one unit to a different unit. This difficulty often necessitates testing different agitation rates in order to approximate the optimized

growth conditions on the original unit. Here we provide information that allows users to easily move from one shaker to a new shaker by converting settings from those optimized on the first unit to equivalent settings on the new unit. This saves time and resources by eliminating empirical testing to determine optimal settings on the new shaker.

## Growth and Metabolism are Controlled by Dissolved Oxygen

When microorganisms are propagated for growth and protein production, oxygen must be provided; otherwise they will die or switch to anaerobic growth, which would produce different results, e.g., alcohol, carbon dioxide gas, and acid production. For aerobic and facultative aerobic bacteria, their rate of growth and metabolism depend upon the amount of dissolved oxygen available.[1] Oxygen has only limited solubility in liquids. Because oxygen does not dissolve very well in liquid, when propagating bacteria in liquid culture, the liquid must be aerated to provide dissolved oxygen.[2] The best aerobic bacterial growth is limited by the speed at which the microbes can replicate, and by the speed at which oxygen gas can be dissolved in the liquid culture medium. Unless oxygen can be pumped directly into the liquid culture, the best way to provide aeration is by vigorous stirring or shaking of the culture flask.

## Too Much Volume Reduces Oxygen Transfer

Bacteria are grown on orbital shakers in flasks of varying sizes. Best results are obtained when the culture volume is approximately 10-25% of the flask volume.[3] This is because a smaller volume will maximize the surface area exposed to the air. Thus, for a 2 liter flask, 200-500 milliliters of growth medium are recommended. Baffles in flasks are not recommended since they create too much splashing.[3] Splashing creates foam which can overflow the flask, or allow contamination of the culture.

## Orbital Shakers: Relationship to Oxygen

In terms of agitation, there are two key variables of the orbital shaker. One key variable that depends upon the individual shaker unit is the diameter of the orbit that the shaking platform describes. Most orbital shakers have an orbit diameter from 0.5 inch (1.27 cm) to 2 inches (5.1 cm). The size of the orbit will affect how the bacterial cells circulate and how the growth medium is aerated. It turns out that shaker orbit diameter has a linear relationship to the Oxygen Transfer Rate (OTR)[4], which is the rate at which oxygen gas is transferred from the air to the liquid. Thus, if the orbital shaker diameter doubles, so does the OTR.


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Similarly, OTR has a roughly 1:1 relationship to the other key variable of the orbital shaker, which is agitation speed.[5] Using a 250 mL flask with 26 mL volume and a 2 inch (5 cm) orbit diameter, Maier and Büchs demonstrated that at 150 revolutions per minute (RPM), they achieved a maximum OTR of 11, while at 300 RPM the maximum OTR was 20.5 It is important not to increase the agitation rate too much, because too much agitation can create shear which can damage bacterial cells.

## Acceleration is Due to Centrifugal Force and Velocity

When shaking bacteria in a flask in order to provide oxygen, we are mostly concerned with the surface area of the liquid because that interface is where the oxygen dissolves from the air into the liquid. By shaking the flask, we cause the liquid to wash up the sides of the flask, which, when done at the proper speed, creates more liquid surface area. In increasing the surface area exposed to the air, we increase the oxygen transfer to the liquid.

When moving from a shaker to a new shaker with a different orbit, the goal is to establish a new agitation speed that will create the same force and resulting liquid movement washing up the sides of the flask as on the original shaker. Newton's second law of motion states that force = mass x acceleration. The mass is the same on each shaker if the same flask and same volume of liquid are used. The acceleration for each flask is equal to the velocity$^2$/radius[6]. Since the radius is half the orbital diameter and velocity equals the agitation speed (in RPM) multiplied by the circumference of the orbit, we can solve for the new agitation speed for the new shaker.

Cancelling the same terms on each side of the equation yields the following formula:

$$r_2 = \sqrt{\left(r_1^2 \times \frac{d_1}{d_2}\right)}$$

Here, $d_1$ = the orbital diameter for the old shaker, $d_2$ = the diameter for the new shaker, $r_1$ = the RPM for the old shaker and $r_2$ = the RPM for the new shaker.

This equation can be easily applied to move among orbital shakers with different orbit diameters to achieve similar OTRs. For example, in moving from one shaker with a 1 inch (2.5 cm) orbit and shaking at 300 RPM, a user moved to a new shaker with a 0.75 inch (1.8 cm) orbit and determined a new speed of 350 RPM to achieve a similar OTR. The equation predicts the new speed should be 346 RPM, which nicely agrees with the empirically produced result from the user.

In another example, a user moved from a unit with an 18 mm orbit diameter shaking at 230 RPM to a new shaker with a 25 mm orbit diameter and found 200 RPM gave the best OTR. Our equation predicted 196 RPM. These examples demonstrate that this formula agrees with empirically determined results within 2-3%. Thus, users can use this formula to easily move from one shaker to another, and simply increase or decrease the predicted speed to the nearest round number.

## Conclusion

In converting from one shaker to a different shaker with a different sized orbit, the orbit diameter can be combined with a balanced adjustment of the shaker agitation rate to obtain a given OTR in order to properly aerate a bacterial culture for optimum growth. At the same time the agitation can provide gentle mixing of the culture that does not damage the bacterial cells, which would lead to cell death and loss of desired genetic material or protein. The formula provided in this article allows convenient transfer from an old orbital shaker unit to a new unit. This simple calculation saves time and materials that would be required to experimentally determine the proper speed to produce the same OTR and bacterial growth on the new unit.

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