high speed orbital shaking for aerobic bacterial growth

Stratus plate reader

introduction

The growth of low-volume bacterial cultures is advantageous for reducing material costs and increasing analytical throughput. Standard 96-well plates are often used for bacterial growth experiments, but achieving sufficient oxygen transfer rates for aerobic growth requires rapid sample agitation. Utilizing the Stratus Plate Reader (Cerillo Inc., USA) with the MS 3 Basic (IKA, Germany), this application note details the result of rapidly shaking an *E. coli* K12 cultures within the Stratus Plate Reader to achieve continuously monitored aerobic bacterial growth curves.

materials and methods

An *E. coli* K12 overnight culture grown in minimal media was diluted to a starting concentration of 0.1 OD. The diluted culture was then aliquoted into a standard clear polystyrene 96-well plate at a volume of 200 µL per well. 80 of the 96 wells contained the diluted growth culture, 16 wells contained sterile minimal media, also at a 200 µL volume. The minimal media used contained M9 salts supplemented with 2.7 g/L glucose for optimal *E. coli* K12 growth (Wang, 2018).

Once aliquoted, the microplate was covered with a BreathEasy® film (Diversified Biotech, Inc., USA) to inhibit evaporation. The plate was then placed on the MS Basic shaker set to 200 rpm which was located inside a heated incubator set at 37°C. The experiment ran for 20 hours. Once completed, an identical diluted culture was prepared and the experiment was duplicated at a 500 rpm shaking speed.

results

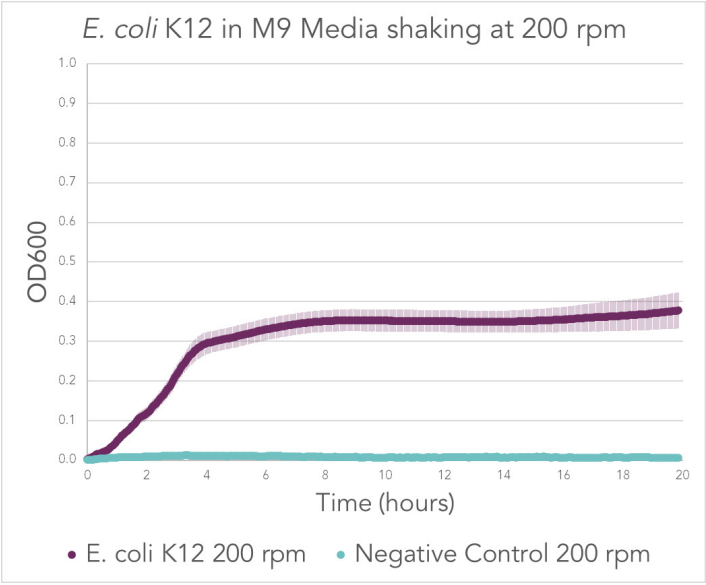

Figure 1. Growth curve results from shaking *E. coli* K12 growth culture at 200 rpm. Error bars indicate standard deviation.

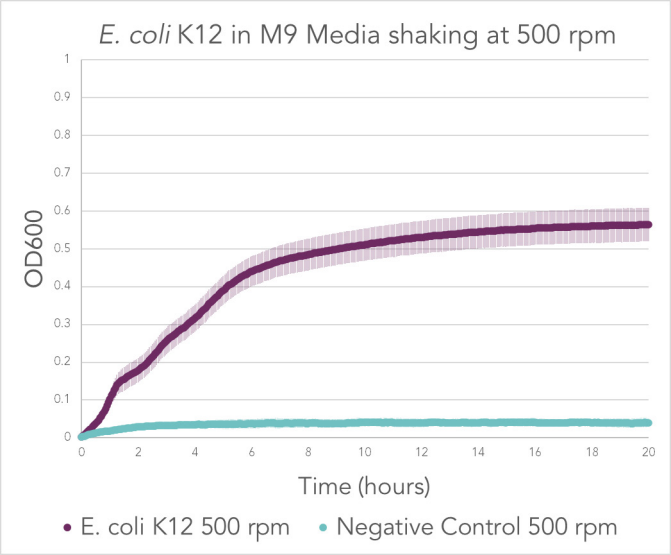

Figure 2. Growth curve results from shaking *E. coli* K12 growth culture at 500 rpm. Error bars indicate standard deviation.

The results show the maximum OD600 reading for the plate shaken at 500 rpm is significantly higher than the plate shaken at 200 rpm. This suggests that the overall biomass yield increased with a more vigorous shaking protocol.

summary

Agitation of bacterial growth samples can have a large impact on both growth rate and biomass production due to increases in oxygen transfer rates for sufficiently agitated samples (Duetz, 2000). When selecting an orbital shaking protocol, visually analyzing the turbulent flow of the sample is imperative to predicting if sufficient oxygen transfer rates will be achieved for optimal growth (Duetz, 2001). When visually analyzing the plates in this experiment, the 200 rpm experiment resulted in far less visual turbulence within the sample wells than the 500 rpm experiment.

It is therefore important when using the Stratus plate reader to first identify the shaking rotation rate required to agitate samples visually before estimating the desired experimental rate. The rotation rate at which turbulent flow is achieved is dependent on a variety of factors including sample volume, shaking radius, microplate well diameter, and angle of rotation (Duetz, 2000). Consideration of all of these factors should be taken when determining the desired rate to achieve ideal microbial growth.

references

Duetz, Wouter A., Rüedi Lorenz, Robert Hermann, Kevin O'Connor, Büchs Jochen, and Bernard Witholt. "Methods for Intense Aeration, Growth, Storage, and Replication of Bacterial Strains in Microtiter Plates." *Applied and Environmental Microbiology* 66, no. 6 (2000): 2641–46. https://doi.org/10.1128/aem.66.6.2641-2646.2000.

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