# Growth Rate, Metabolite Production, and Final Biomass in a Tumble Stirred Culture Vessel

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## Introduction

A crucial aspect of aerobic growth of bacteria is the mass transfer of oxygen into the solution. A simple and effective way of implementing this is through rigorous stirring of the solution. In the context of a small number of large culture vessels (>100mL) this can be accomplished through a traditional magnetic stir bar placed in the vessel with a rotating magnet underneath the vessel. However, as the culture gets smaller (<50mL) and number of cultures increases, it no longer becomes feasible to have a stirring platform with 1 magnet per stir bar for two main reasons. First, the stirring positions are typically spread fairly far apart to eliminate interference of the magnets as well as remaining versatile enough to stir larger vessels. Thus it becomes an inefficient use of space. When trying to grow bacteria in an incubator or a robotic liquid handling environment, space is often at a premium. To obtain efficient packing of the cultures a customized stirring system becomes the best option. This leads to the second downfall which is the lack of versatility. In the case where the size or number of culture vessels needs to be changed, the traditional stirring system cannot adapt in an efficient manner

However, a tumble stirring system allows the user to stir any sized vessel and pack them as loosely or tightly as desired as long as they are within a certain distance of the tumble stirring magnet. However, the tumbling stirring element rotates along a different axis hence the dynamics of the stirring differs. Here we test the ability of a tumble stirring system to stir a culture of *wild-type Escherichia Coli* to maintain the same growth attributes found in a control group.

The experiment tested growth in a control group that consisted of 200mL of media in a 500mL Erlenmeyer flask with a 2 inch stir bar spun at approximately 1400 rpm. Our experimental group consisted of two types of vessels: 50mL polypropylene centrifuge tubes and 50mL Pyrex conical tubes. These tubes were chosen in an effort to minimize the cross sectional area their footprint occupies and to expand the culture vertically to maintain a sufficiently large culture (40mL).

The growth characteristics of each group were compared across 3 categories: exponential growth rate, final biomass, and metabolite production. Each of these categories is sensitive to the mass transfer of oxygen into the media. Five different tumble stirring speeds were tested with triplicates of each variety.

## Materials and Methods:

**Culture Vessels.** Control experiments were performed in a 500mL Erlenmeyer flask filled with 200mL of M9 minimal media with 4g/L D-glucose, trace elements, and a 2 inch magnetic stir bar. The culture was stirred at approximately 1400rpm. The experimental vessels were filled with 40mL of M9 minimal media with 4g/L D-glucose, trace elements, and a 24mm diameter stir disk (VP 772FN-24-24) and stirred with an alligator stirring system (VP 710). The speeds tested at were 20, 40, 60, 80, and 100 which are arbitrary units marked on the speed control knob on the tumble stirrer. Each vessel was incubated for more than 6 hours in a 37ºC incubator before being inoculated. This study was performed in triplicate.

**Inoculation.** *Wt-E. Coli* was grown in a 500mL Erlenmeyer flask filled with 200mL of M9 minimal media with 4g/L D-glucose, trace elements, and a 2 inch magnetic stir bar stirred at approximately 1400 rpm. The growth rate was monitored (see below) and during mid exponential phase the control and experiment vessels were inoculated. The vessels were then incubated in 37ºC incubator.

**Growth Rate Monitoring.** The growth rate was determine by sampling <1mL of the solution and taking an optical density reading at 600nm ($OD_{600}$). The sample was diluted below 0.4 to obtain a linear relationship between $OD_{600}$ and biomass. The first sample was taken at least 12 hours after inoculation to avoid sampling during the lag phase. Growth rate was determined by a minimum of 3 samples.

**Final Biomass.** The final biomass measurement was obtained after no appreciable increase in $OD_{600}$ was observed.

**Metabolite Measurement.** After the final biomass measurement was taken, a >2mL sample of the culture was filtered through 33mm Millex-GP PES filter. The concentration of metabolites in the filtered solution was obtain by high throughput liquid chromatography and compared to a standard curve of known sample concentrations.

## Results and Discussion

The growth rates at each speed were found to increase with an increase in the tumbling speed (figure 1). At a tumbling speed of 80, the growth rates of both experimental groups match the control. At a tumbling speed of 100 the growth rate in the Falcon tube did not statistically match the control, however, the stir discs would often be uncoupled from the magnetic field and would only twitch and not tumble over. The growth rates at 20, 40, and 60 did not exhibit purely exponential growth. This is indicative of mass transfer issued; in this case, oxygen. When the media is first inoculated, the partial pressure of oxygen (pO2) is at a maximum. As the biomass and total oxygen consumption remains low, it is able to grow at its maximum rate. With an increase in biomass comes and increase in oxygen consumption. Without adequate oxygen mass transfer the pO2 will decrease and the maximum allowable growth rate along with it.

The metabolite production showed an inverse relationship between total metabolite concentration and tumble stirring speed (figure 3). In the presence of adequate oxygen, wt-E. Coli will primarily oxidize glucose into carbon dioxide as this allows for the most efficient utilization of glucose for energy. In the presence of decreased pO2, carbon dioxide cannot be formed in stoichiometric ratio to the influx of glucose. Usable energy can still be obtained from glucose without oxygen available, just not with the same efficiency. These alternative pathways produce by products other than carbon dioxide. Wt-E. Coli tends to produce ethanol, succinate, D-lactate, acetate, and formate in varying amounts. We can then see that at speeds of 20, 40, and 60 metabolite productions is elevated beyond the control.

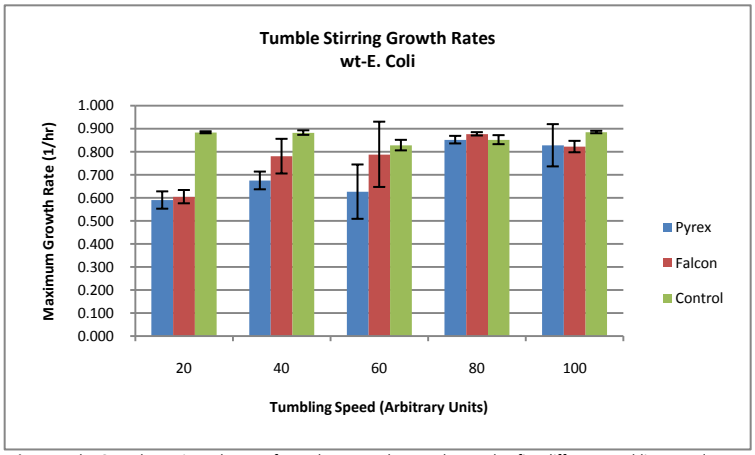


**Figure 1:** The Growth rate in each type of vessel, Pyrex, Falcon, and control at five different tumbling speeds. There is a significant difference between the control and experimental groups for speeds 20, 40, and 60. Stirring at 80 showed the most consistent results when compared to the control. While stirring at 100 the stir disc would often become uncoupled from the spinning magnetic field. Without first slowing down the tumbling speed, the stir disc would remain uncoupled. Hence, the average growth rates as well as the standard error are not as closely matched with the control compared to stirring at 80. The growth at 20, 40, and 60 did not exhibit purely exponential growth.

The final biomass also shows signs of poor oxygen transfer at speeds 20, 40, and 60 (figure 3). Since no additional nutrients are added to the culture over its growth duration the final biomass should have a relation to the total energy and mass available. The metabolites produced by wt-E. Coli are higher in energy than carbon dioxide thus the total energy available to use by the bacteria is less under low pO2 condition. Indeed the slower speeds show indications of poor mass transfer of oxygen.

Based on the relative differences of each of the three characteristics measured, the falcon tube outperformed the Pyrex tube. These differences are most likely due to the position that the stir disc rotated in each vessel. The Pyrex tube has a much less aggressive conical shape than that of the Falcon tube. Thus the stir disc rotated along an axis that was much high in the vessel. Obviously the stirring in each of the tubes showed distinct differences that may be attributed to the position of the stir disc or the overall shape of the vessel.

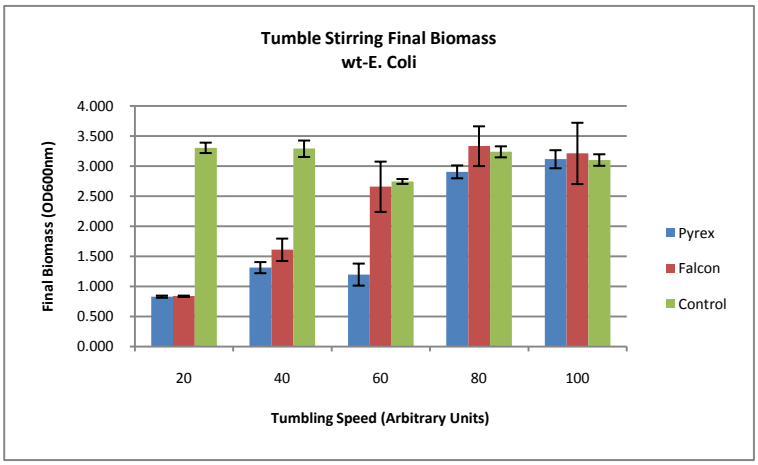


**Figure 2:** Final Biomass measured as the optical density at 600nm ($OD_{600}$) in each type of vessel, Pyrex, Falcon, and control at five different tumbling speeds. The Pyrex tube is significantly less at all tumbling speeds except 100. The Falcon tube is not significantly different for speeds at 60, 80, and 100. However, the control experiment at 60 is the lower than the other controls.

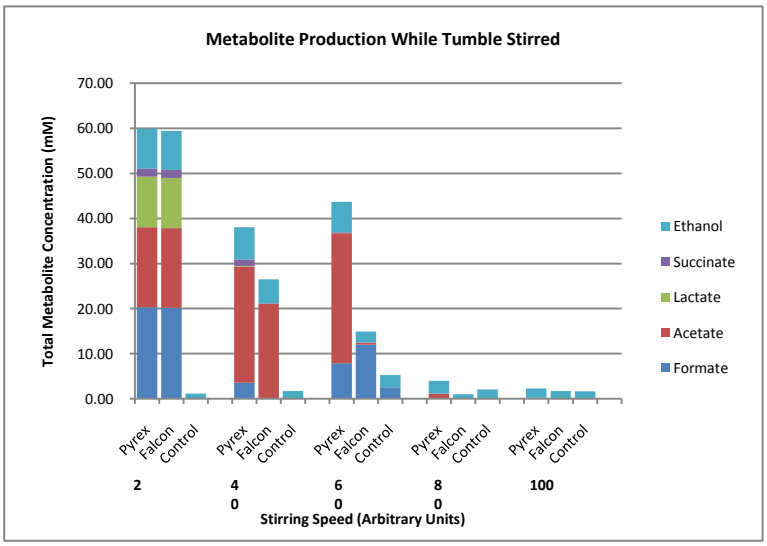


**Figure 3:** Concentration of ethanol, succinate, D-lactate, acetate, and formate at endpoint. Stirring at speeds 20, 40, and 60 show significantly more metabolite production than the control. This is indicative of a lack of mass transfer of oxygen into the media. With excess oxygen present the E-coli will prefer to make carbon dioxide (not measured).

## Conclusion

The growth of *wt-E. Coli* was observed under tumble stirring conditions in two different vessels, 50mL polypropylene Falcon tubes and 50mL Pyrex glass conical tubes at five different tumbling speeds. All these were compared to a control group. Three characteristics of growth were observed in each culture: growth rate, final biomass, and metabolite production.

Based on these characteristics it was found that at speeds of 20, 40, and 60 (arbitrary units) there were signs of poor mass transfer of oxygen into the culture. This was indicated by a lower growth rate and final biomass, as well as an increase in metabolite production when compared to the control. At speeds of 80 and 100, the growth rates, final biomasses, and metabolite production matched with the control experiment, except the final biomass of the pyrex tube at a speed of 80.

It was observed that at a speed of 100 the stir disc could become uncoupled from the magnetic field. This is an unfortunate consequence as stirring more rigorously is generally better than under stirring with an organism with a strong cell wall. No uncoupling of the stir bar was observed at speeds other than 100.

Overall, the tumble stirring system was able to sufficiently aerate a 40mL culture of *wt-E.Coli* such that no appreciable difference could be observed when compared to a control. The suggested vessel and tumbling speed would be the use of the 50mL Falcon tube at a speed of 80. It slightly outperformed the Pyrex tube and at a speed of 80 no uncoupling observed.

| Stirring Speed (Arbitrary Units) | Rotations per Minute |
|---|---|
| 20 | 123 |
| 40 | 388 |
| 60 | 641 |
| 80 | 898 |
| 100 (max) | 1140 |

**Figure 4.** Tumbling stirring speeds as a function of the arbitrary units located on the speed control knob. These speeds were measured using a stroboscope.