

**EFFECT OF VIBRATION ON BACTERIAL GROWTH AND ANTIBIOTIC
RESISTANCE**

SUMMARY OF RESEARCH

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October 29, 2004

EFFECT OF VIBRATION ON BACTERIAL GROWTH AND ANTIBIOTIC RESISTANCE

FOREWORD

This report describes the research performed under the grant NAG2-1512, during the period of August 2001 to October 2004 (grant termination). Elizabeth A. Juergensmeyer, Ph.D. was the Principal Investigator for this grant. This report was prepared by Elizabeth A. Juergensmeyer and Margaret A. Juergensmeyer.

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EFFECT OF VIBRATION ON BACTERIAL GROWTH AND ANTIBIOTIC RESISTANCE

SUMMARY

The purpose of this research grant was to investigate the response of bacteria to vibrational acceleration. Over the three years of this grant, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were exposed to linear vibration of a known frequency and amplitude. The cells were grown in either hard-sided containers (syringes) or soft-sided containers (bags). Growth was monitored by optical density readings and, on occasion, plate counts. Growth curves indicated that vibrational acceleration induces changes in the growth curve of the organism, including reduced lag time and increased growth rate. Oxygen consumption within the containers was measured, and results indicated that the container had no effect on oxygen consumption, while vibration did. The response of bacteria to vibration is similar to the response seen to spaceflight, indicating that vibration on an orbital platform could be a significant factor in the microgravity environment.

I. OBJECTIVE

The purpose of this research grant was to provide a fundamental, systematic investigation of the effects of oscillatory acceleration on bacterial proliferation and their responses to antibiotics in a liquid medium.

II. MATERIALS AND METHODS

A. Vibrational Acceleration

Linear acceleration was produced by reciprocating shaking water baths. Two baths were used: a VWR Scientific Products bath (VWR, Chicago), and a Precision bath (Fisher Scientific, Chicago). The stroke length of both devices could be varied at ½”, 1”, and 1 ½” (13, 25 and 38mm) but was left at 1” (25mm) except for specific experiments, and the frequency was set at a range from 20-60 rpm, or 0.33 and 1 Hz.

B. Hardware Devices

In order to simulate both the hard-sided and soft-sided hardware seen in spaceflight, two different containers were used. For hard-sided containers, 10-ml syringes (VWR, Chicago) were filled with a total of 10 ml of the bacterial cultures. For soft-sided containers, 10 ml of the bacterial culture was dispensed into Whirl-Pak bags (VWR). All air was evacuated from both the bags and syringes, which resulted in a very similar aspect ratio (height and diameter of fluid column) for both. The bags were then attached to the syringes by clips, producing a single “hardware device” comprised of one bag and one syringe, which could then be treated as a single unit.

C. Optical Density

Growth of the cultures in broth was monitored by optical density (OD) readings. At intervals throughout the course of the experiment, hardware devices were harvested, and the cultures dispensed into cuvettes. The cuvettes were inserted into either a Spectronic-20D+ (Fisher Scientific, Chicago) or a Biophotometer (VWR, Chicago), and the OD recorded. A total of five hardware devices were harvested at each time point, to increase statistical significance.

D. Dissolved Oxygen

The concentration of oxygen in both container types was measured over the timecourse of a typical experiment. A container (bag or syringe) would be filled with 10 ml of the bacterial culture, and the dissolved oxygen probe (Accumet, Fisher Scientific) was inserted. All bubbles were removed from the container, and the probe was sealed into the container using parafilm. The container was then placed into the water bath, and observed until the readout on the dissolved oxygen meter stabilized. This was considered to be Time 0, and recording began at this point. The shaker of the water bath was then either left stationary or turned on for vibration. Dissolved oxygen concentration was measured automatically every minute, and recorded automatically.

E. Oxygen Access

To further investigate the requirements of oxygen within the system, syringes were modified to allow limited access to oxygen. Some syringes were loaded with 5 ml of culture and 5 ml of ambient air, while others had the plunger removed and replaced with a sterile foam stopper. The foam stopper allowed access to ambient air without the risk of contamination.

F. Antibiotic Resistance

E. coli cultures, grown as described above, were challenged with a suite of 12 antibiotics to identify any changes in antibiotic resistance. The cultures were inoculated and incubated as described. At various timepoints, the cultures from the four experimental conditions (shaken in syringes, shaken in bags, stationary in syringes, stationary in bags) were harvested and spread-plated onto Mueller-Hinton (MH) agar. The plates were allowed to dry for 5-15 minutes, and antibiotic disks (Fisher Scientific) were dispensed onto the plates. The disks contained one of the following antibiotics: ampicillin, cephalothin, chloramphenicol, colistin, erythromycin, gentamycin, kanamycin, polymyxin B, penicillin G, tetracycline, rifampicin, or streptomycin. Three disks were placed on a single agar plate, to ensure sufficient room to measure growth.

The plates were inverted and incubated overnight at approximately 37°C. After incubation, the zone of inhibition (ZOI) around each disk was measured and recorded.

III. RESULTS

E. coli

Figures 1 and 2 show the response of *E. coli* to vibration, at 0.33 and 1.0 Hz respectively. In syringes, vibration induces changes in the growth curve, including decreased lag time, increased growth rate, and earlier plateau as compared to simultaneous stationary controls. These effects are seen at both Hz. Cultures grown in bags show a very different growth curve than those grown in syringes. Bag cultures show a slightly higher growth rate and a higher final OD, and a later plateau time. In bags, however, there is no effect of vibration on the growth curve of the culture. Changing the frequency of vibration has no effect on the growth curves.

Effect of Oxygen

Figure 3 shows the concentration of dissolved oxygen over time as *E. coli* grows in either syringes or bags, with and without vibration. While the rate of consumption changes slightly between shaken and unshaken containers, it is of interest to note that in all containers, the available oxygen in the medium is completely consumed within 90-120 minutes. This is approximately the end of the lag phase, which indicates that oxygen consumption has little effect on the later stages of the growth curve. In addition, the bags do not appear to be oxygen-permeable. Since *E. coli* is a facultative anaerobe, the lack of oxygen would not be expected to affect its ability to grow.

Figure 4 compares the growth curves of *E. coli* grown in syringes only, with and without a bubble of ambient air. Growth curves at 0.33 Hz values are presented, and demonstrate that the presence of air in vertical syringes has no effect on the response to vibration. No difference in response was seen by varying the frequency. These data were collected using syringes shaken in the standard vertical orientation (see Fig. 6), while Figure 5 demonstrates that syringes shaken while in the horizontal orientation show slight response to the presence of ambient air bubbles. Overall,

vertically oriented and shaken syringes show similar responses to vibration with or without oxygen. Intriguingly, the stationary syringes show a response to the presence of additional oxygen, as seen by an earlier log phase, later plateau, and increased cell number. Syringes shaken while in the horizontal orientation, however, show a delayed response to oxygen. Whether shaken in the X or Z axis, the cultures grown in a horizontal orientation and shaken show an early log phase and a growth rate similar to the other shaken syringes. Only after 3 hours is there a change between cultures with no air or cultures shaken vertically with air, both of which plateau; cultures shaken in the X axis, which continue in log phase for another 2 hours; and cultures shaken in the Z axis, which also continue in log phase for 2 hours, but show an increased cell number.

Antibiotic Resistance

E. coli cultures in shaken syringes show an increased susceptibility to Colistin, as compared to unshaken syringes, but do not change in response to any other antibiotic tested. These same cultures grown in shaken bags show increased susceptibility to Colistin, Gentamycin, and Polymyxin B, as compared to unshaken bags. None of these changes fall within the range required for clinical susceptibility; they merely indicate an increase in susceptibility (data not shown).

S. aureus

Figure 7 shows the response of *S. aureus* to vibration in syringes. As seen in *E. coli*, the lag phase of shaken *S. aureus* is reduced, and the growth rate of the shaken cultures is higher than that of stationary controls. The presence of excess oxygen, as seen by the syringes with foam stoppers, enhances growth during the mid-log phase of the growth curve, but has no effect on the reduced lag phase seen in the shaken cultures.

P. aeruginosa

Figure 8 shows the growth curve of *P. aeruginosa* when exposed to vibration. This organism grows very poorly in the absence of oxygen so the media was supplemented with sodium nitrate as an alternate electron acceptor, allowing anaerobic growth.

Instead of investigating growth in bags, only syringes were used on this culture. Overall, the effect of vibration is similar to that seen in other cultures. Vibration decreases the lag phase, particularly in the cultures exposed to ambient air. It should be noted that a plateau in OD is not expected for this culture, as it produces a polysaccharide “slime” throughout its life cycle. Even after the cell number has stabilized, the polysaccharide will continue to increase, and cause an increase in the OD readings.

B. subtilis

Figure 7 shows the response of *B. subtilis* to vibration. Again, vibration induces a shorter lag phase both in cells with access to atmospheric oxygen and in cells in sealed containers. *B. subtilis* is more of an obligate aerobe than *E. coli* or *S. aureus*, and it is quite evident that the presence of oxygen enhances growth throughout the timeline. However, vibration alone results in a sharp reduction in lag phase, from approximately 5 hours in a non-shaken culture to only two hours in a shaken culture. The extended lag phase is seen in stationary cultures regardless of the presence of oxygen, which only begins to impact growth after approximately 6 hours.

IV. DISCUSSION

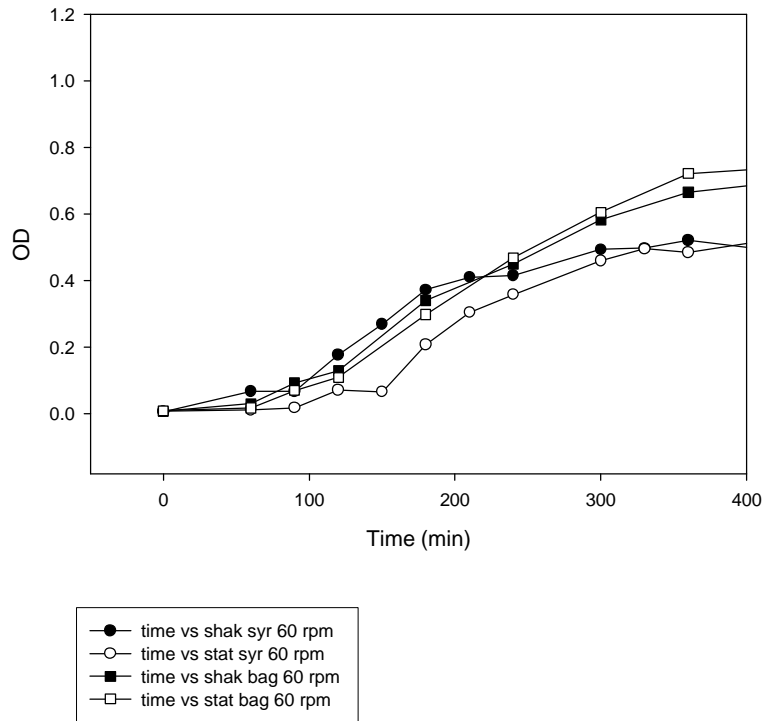
Vibration, without concurrent oxygenation, affects the growth of bacteria. In all species tested (*E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*), vibration was able to decrease the lag phase and increase the growth rate of the cells. Addition of oxygen to the cultures enhanced this effect, but removal of oxygen did not negate the effect. This response is only seen in hard-sided containers; cultures grown in soft-sided containers show no response to vibration. These results indicate that the changes in growth observed in space flown cultures may be due to a subset of the microgravity environment, such as vibration, rather than being directly attributable to microgravity. They also indicate that the choice of hardware can influence the results of experiments.

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V. FIGURES

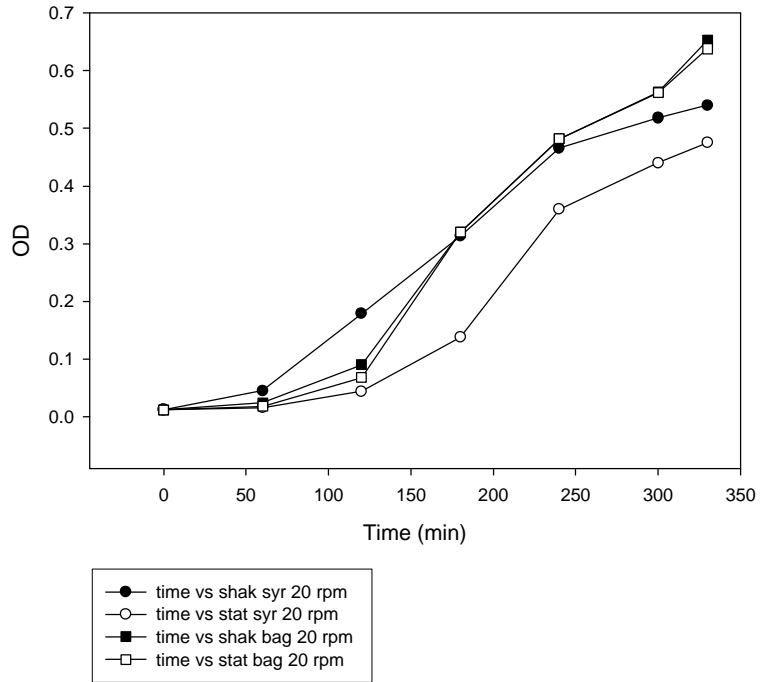
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Figure 1: Growth of *E. coli* in bags and syringes, exposed to 1Hz vibration



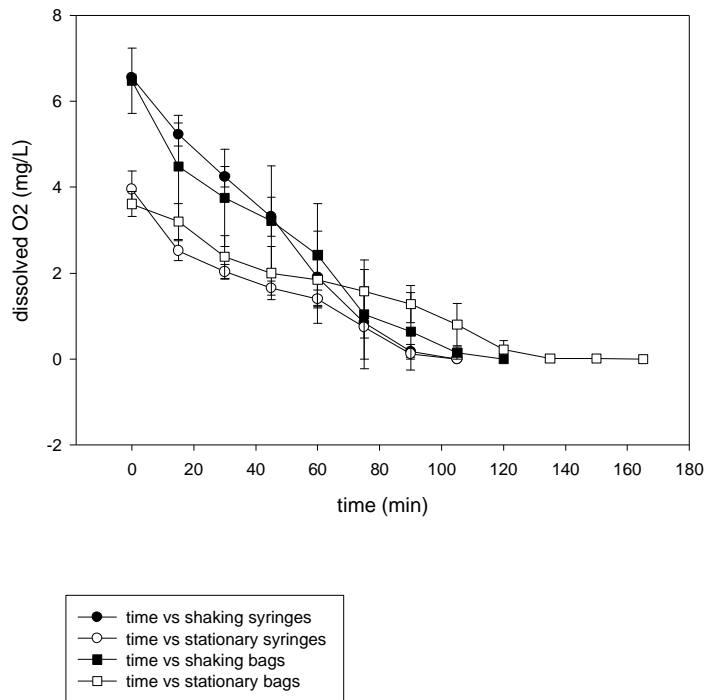
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Figure 2: Growth of *E. coli* in bags and syringes, exposed to 0.33Hz vibration



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Figure 3: Dissolved oxygen concentration in bacterial cultures exposed to vibration, in both bags and syringes



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Figure 4: Growth of *E. coli* in syringes, with and without 5 ml ambient air, with and without vibration at 0.33 Hz.

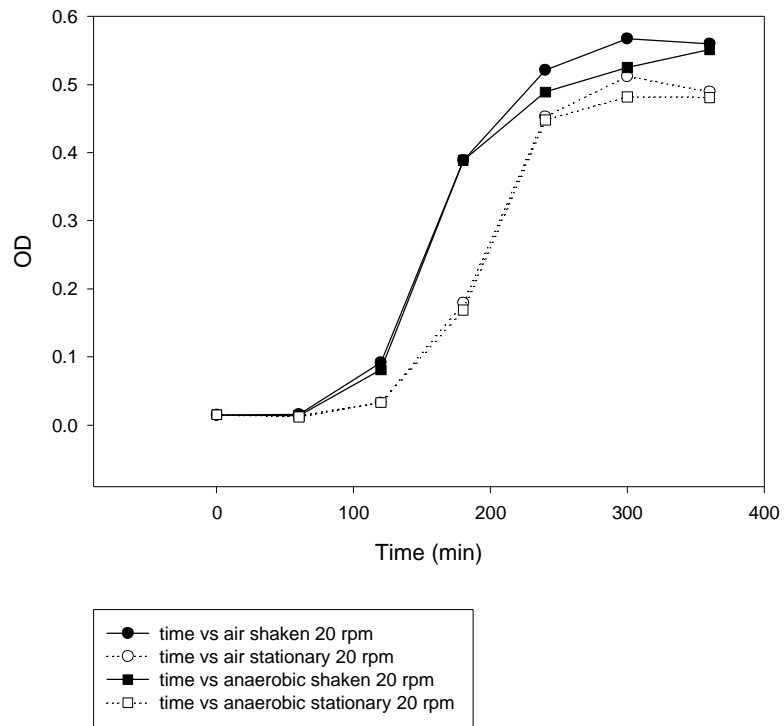
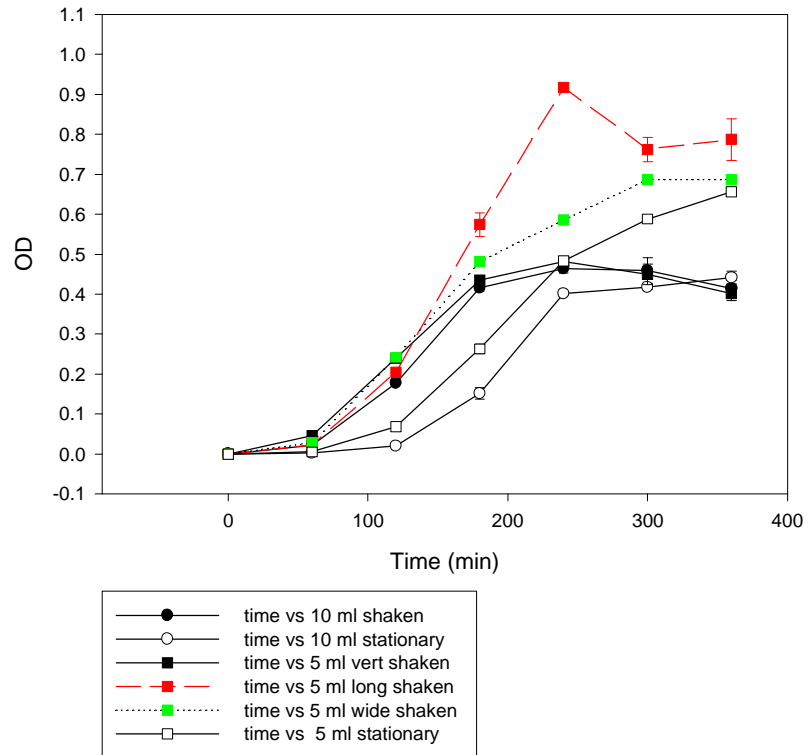


Figure 5: Effect of vertical and horizontal shaking on *E. coli* grown in syringes, with and without 5 ml air



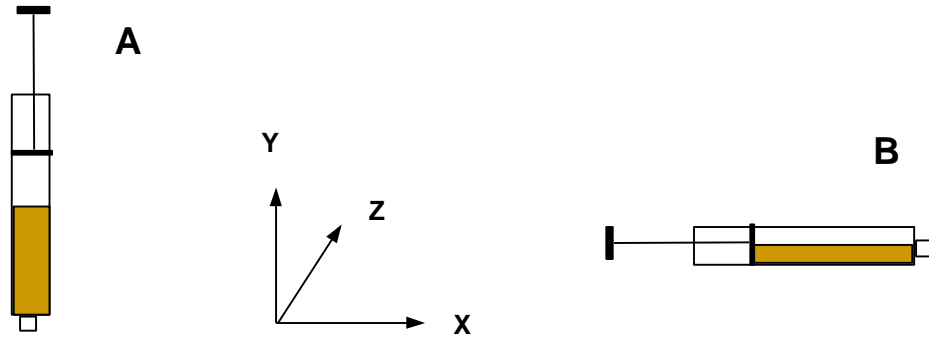


Figure 6: Orientation of shaken syringes.

Unless otherwise noted, syringes were placed vertically (A) into the shaker bath, and shaken along the X axis. When air bubbles were added, however, the effect of increased air/liquid surface contact was tested by placing the syringes in the shaker bath in a horizontal configuration (B). These syringes were shaken along both the X and Z axes.

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Figure 7: Effect of vibration on *S. aureus* in sealed syringes and exposed to ambient air

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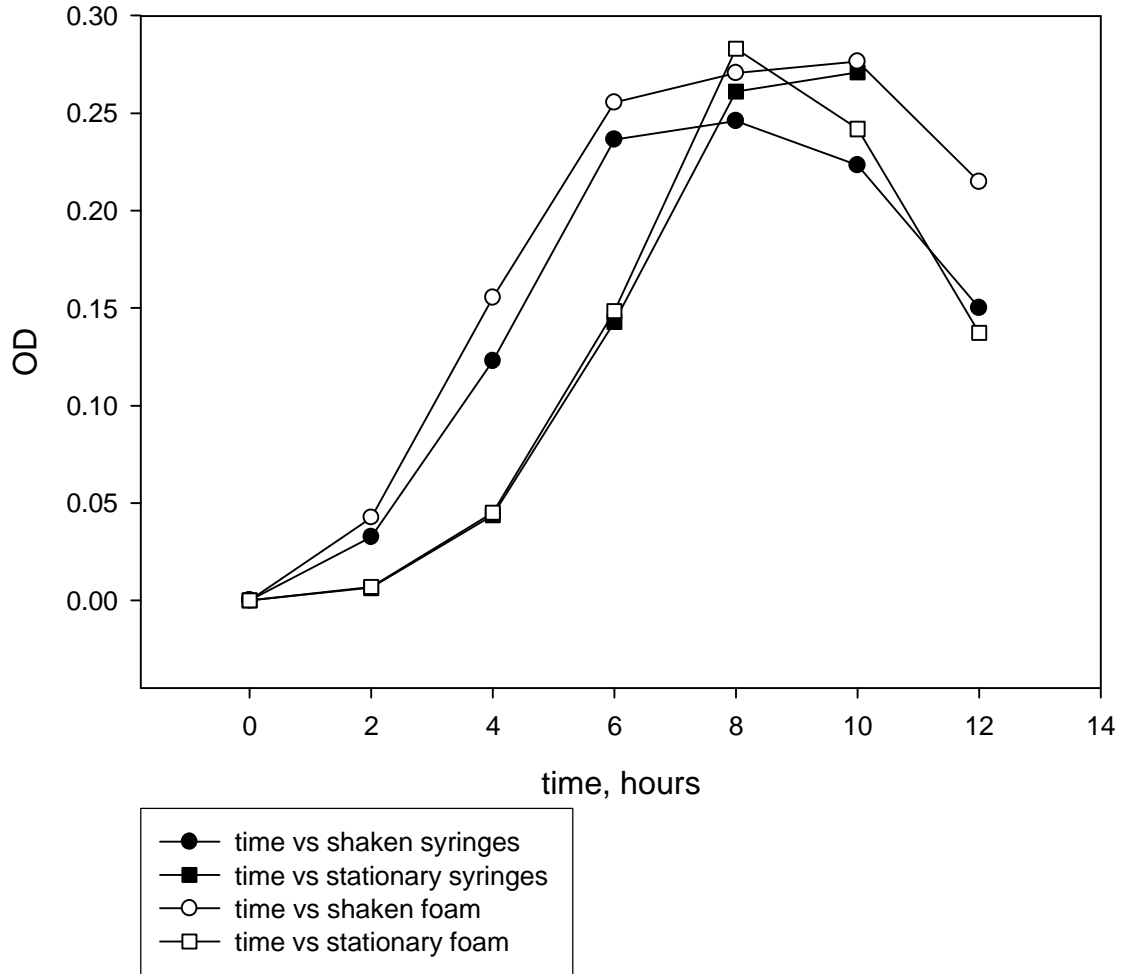
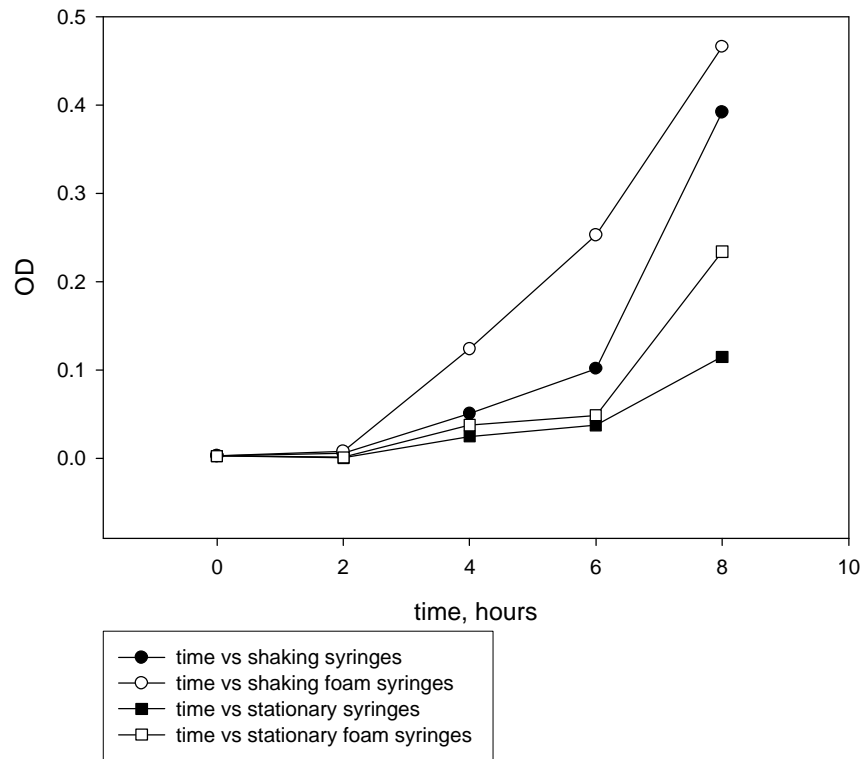
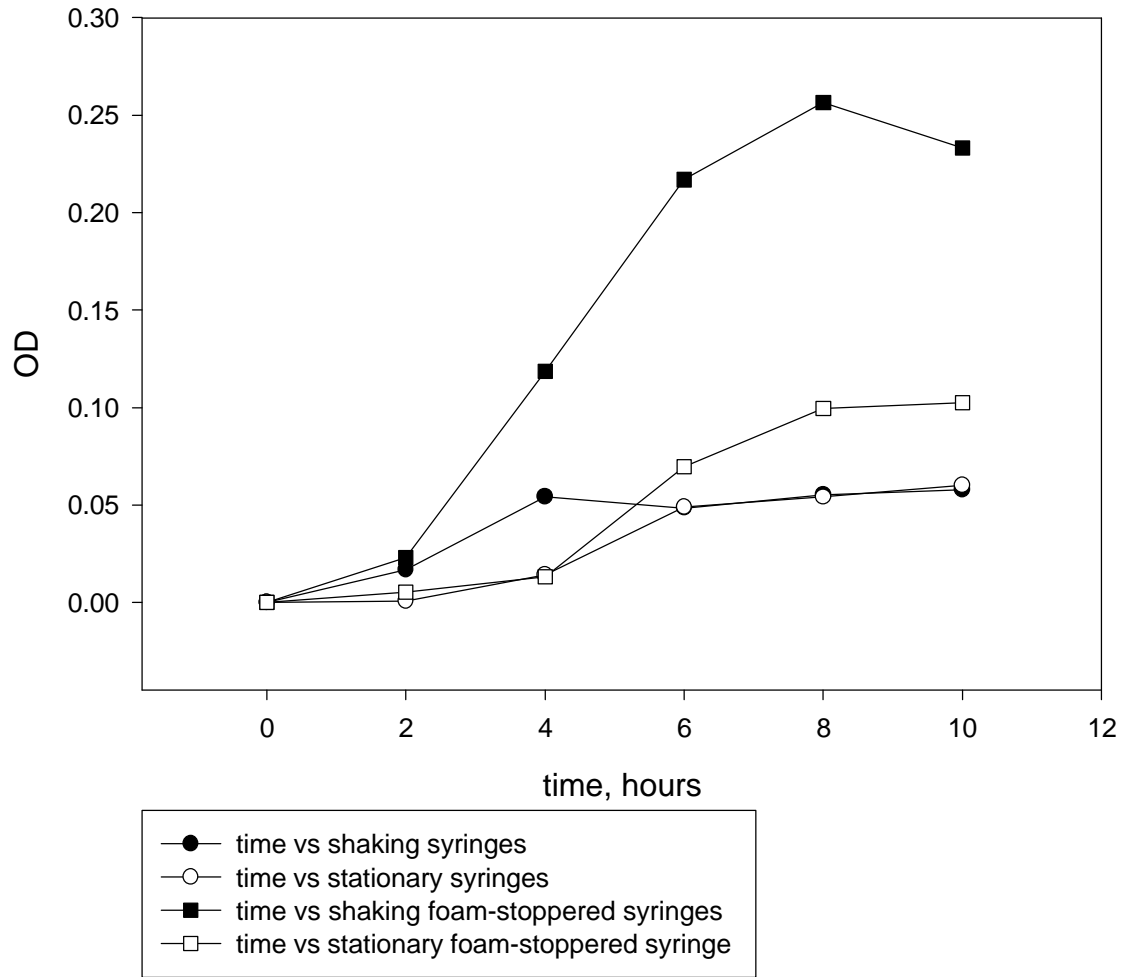


Figure 8: Effect of vibration on *P. aeruginosa*, in sealed syringes and with exposure to ambient air



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Figure 9: Effect of vibration on *B. subtilis* in sealed syringes and with exposure to ambient air



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VI. APPENDICES

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Appendix A

Presentations

2002 – Effects of Vibration on Bacterial Growth Rate and Similarities to Space-Flown Cultures. E.A. Juergensmeyer, M.A. Juergensmeyer, Rachel N. Edes, E.S. Nelson. Poster presentation at American Society for Gravitational and Space Biology.

2002 – Effects of Shaking without Aeration on *Escherichia coli* Grown in Liquid Media. Rachel N. Edes, E.A. Juergensmeyer, M.A. Juergensmeyer, E.S. Nelson. Poster presentation at American Society for Gravitational and Space Biology.

2003- Effects of Vibration on Antibiotic Resistance in *E. coli*. M.A. Juergensmeyer, E.A. Juergensmeyer, and E.S. Nelson. Poster presentation at American Society for Gravitational and Space Biology.

2004- Effects of Vibration on Bacterial Growth. E.A. Juergensmeyer, M.A. Juergensmeyer and E.S. Nelson. Poster presentation at American Society for Gravitational and Space Biology.

Publications

Shaking Alone, Without Concurrent Oxygenation, Affects the Growth Rate of *E. coli*. Margaret A. Juergensmeyer, Elizabeth A. Juergensmeyer, and Emily S. Nelson. In press.

Patents

No patentable items, information or inventions were produced by this project.