The Effect of Magnetic Fields on the Quorum Sensing-Regulated Luminescence of *Vibrio fischeri*

Addie Barron\(^1\), Dr. Steve Hagen\(^2\), Dr. Minjun Son\(^2\), Delaram Ghoreishi\(^2\)

\(^1\) Department of Physics, Centenary College, Shreveport, LA 71104, U.S.A

\(^2\) Department of Physics, University of Florida, Gainesville, FL 32611, U. S. A.

**Abstract**

Quorum sensing (QS) is the mechanism by which the bioluminescent marine bacterium *Vibrio fischeri* is able to communicate with other cells of the same species. While there is much known about the mechanism of quorum sensing, little is known about the factors that affect it. Here we report the effects of a relatively high magnetic field on the QS-regulated bioluminescence of *V. fischeri* using a different method than previous studies. *V. fischeri* were grown with photobacterium growth media and subjected to a magnetic field, then imaged over a forty-eight hour period to monitor the bioluminescence. The images were analyzed and the intensity was plotted against time. After analysis of the images and graphs, our results find no evidence that a strong magnetic field influences the luminescence output of *Vibrio fischeri*. 
**Introduction**

Quorum sensing (QS) is a mechanism by which bacteria communicate through the secretion and detection of extracellular signaling molecules known as autoinducers [1, 2]. While the mechanism of quorum sensing is relatively well understood, the effect of environmental factors is not [3]. Quorum sensing regulates virulence, a microorganism’s capability to cause disease, in many human and plant pathogens [2]. By learning more about the factors that affect quorum sensing, we will be better equipped to manipulate the behavior of bacteria, whether they are beneficial or harmful to mankind.

This research project focuses on quorum sensing in *Vibrio fischeri*, a bioluminescent marine bacterium that lives in symbiosis with certain fish and squid species [4]. *Vibrio fischeri* is an excellent species to study because its symbiotic relationship with marine life is a common interaction in nature where host epithelial tissues contain extracellular, organized bacteria [4]. In quorum sensing, each bacterial cell produces a small molecular signal, known as an autoinducer [2], and releases it into the environment. Other *Vibrio fischeri* cells detect that signal as it accumulates. When the concentration of the autoinducer exceeds a threshold level, a signal transduction cascade is initiated that leads to the production of luciferase [2]. This enzyme catalyzes light production in *Vibrio fischeri* [2, 4]. At this point, the concentration of the signal molecule in the environment is large enough that all of the cells switch on their bioluminescence, which is the production of visible light [1].

Previous research [1, 3, 5] has supported the presence of magnetosomes [1,3,5] in *Vibrio harveyi* that give aid to their magnetoreception, the ability of the bacteria to sense magnetic fields [1]. Magnetosomes have been characterized as magnetic organelles [1]
that consist of magnetite (Fe₃O₄) or greigite (Fe₃S₄) crystals bound by a membrane [5]. It has been speculated that these organelles aid in orienting cells to Earth’s magnetic field [1]. The discovery of magnetosomes led to research conducted on how a magnetic field would affect *V. harveyi*’s quorum sensing [1,3].

However, these various studies have conflicting conclusions. There are some that testify to the fact that a strong magnetic field has a positive effect on *V. harveyi* bioluminescence [1, 3], resulting in an increase in intensity, while others attest to a negative effect on various bacteria’s colony-forming units [6]. There has only been speculation concerning the biological effects of magnetic fields, one of which considers how the permeability of a membrane’s ionic channels are affected by a magnetic field, which could impact the cell’s transport system, therefore disrupting its stable state [6]. Overall though, the effect of magnetic fields on quorum sensing-regulated luminescence is still poorly understood. *V. fischeri* is also a bioluminescent marine bacterium like *V. harveyi* [1], leading us to consider the effects of a magnetic field on *V. fischeri*’s QS. The goal of this research project is to confirm or disprove that *Vibrio fischeri* bioluminescence is increased by strong magnetic fields by following a similar procedure performed by previous research projects [1, 3].

**Materials and Methods**

*Preparation of Cells and Growth Media*

A single strain of MJ11 *Vibrio fischeri* was studied in this project. Starting from frozen glycerol stocks of the bacteria, the surface of the glycerol stock was scraped using a syringe needle and placing the entire needle into approximately 12.5mL of photobacterium liquid growth media. The cultures were grown at room temperature
overnight. The optical density (O.D.) of the suspension was measured using a Shimadzu UV-1601 UV-Visible spectrophotometer and the solution was diluted to maintain an O.D. of roughly 0.3. With this suspension, a 1:50 serial dilution was performed; the third dilution (a 1: 2.5 × 10³ ratio) was determined to be the best concentration for our observation. From this dilution, 22µL were placed on a 0.75% photobacterium agar plate and streaked using an L-shaped spreader to achieve uniformity. Liquid cultures were made following the same procedure for measuring the O.D. and performing a 1:50 serial dilution using the third dilution; 8.5µL of this solution was transferred to 8.5mL of growth media in order to achieve a thousand-fold dilution.

Creating the Magnetic Field and V. fischeri samples

To test the effect of a magnetic field on the luminescence, two circular neodymium magnets, each with a 5/16" diameter and 1/8" thickness were mounted onto a glass slide. The magnets provided nominally (according to the manufacturer) a 3170 Gauss surface field. Each was positioned so that the opposite pole would be in contact with the plate; this was done so that some region on the plate has a zero field, although we could not be certain which region exhibited no magnetic field. A 60 mm × 15 mm polystyrene petri dish filled with agar and streaked with Vibrio fischeri cultures was placed on top of the magnets.

Creating the Optical Path

In order to image the 60mm × 15mm plates, we had to devise an optical system that allowed for the entire plate to be imaged in complete darkness so that the bioluminescence could be measured. See Figure 1 for the Optical set-up. To do so, we created an optical system in a black box that omitted any ambient light. The optical setup
consisted of the CCD camera placed on the outside of the box with an aperture in the box to allow the lens to be inserted. We used a lens with a focal length of 2.5 cm and a mirror mounted so that it was at the same level as the lens. Using the lens formula, \( \frac{1}{f} = \frac{1}{d_o} + \frac{1}{d_i} \), where \( f \) is the focal length, \( d_o \) is the distance from the lens to the object, and \( d_i \) is the distance from the lens to the image, we were able to determine the approximate lengths necessary to focus the camera on the plate since we knew the focal length of the lens. For our experiment, we used a \( d_i \) of 5 cm and a total \( d_o \) of 22 cm where the distance from the mirror to the lens was added to the distance from the plate to the mirror (See Figure 1). In order to let the plate lie flat during the trials, a mirror was utilized; by tilting the mirror at approximately a forty-five degree angle, it allowed the reflected image to be collected on the CCD camera.

![Figure 1. Diagram of the Optical Path](image-url)
Procedure for Data Collection

We needed to image the plates at consistent time intervals, so we employed a camera and pulse generator to do so. A Stanford Research Systems, Inc. Model DG 535 Four Channel Digital Delay/Pulse Generator was set to a frequency of 900Hz and connected to a Princeton Instruments Micro Max Charge Coupled Device (CCD) camera and used to generate a periodic pulse that triggered the Micro Max camera to take a picture at time intervals of fifteen minutes with an exposure time of ten seconds. A total of 192 images were collected over a period of forty-eight hours. See Figure 2 for the experimental setup.

Figure 2. A diagram of the experimental set-up.
Analysis of the Bioluminescence

We used two methods to analyze the bioluminescence of the *V. fischeri* cells. The first form of analysis was more qualitative since it involved converting the array of images into a movie. To create a movie, the images were transferred from the Win View software to Matlab where they were compiled into a movie. This was accomplished by converting the file format of the images to one compatible with an avi file. A picture of the magnets was overlaid with the subsequent images, so that the exact location of the magnets could be determined. Their location was marked using circles on the video, which allowed for qualitative observations concerning the effect of the magnetic field on the *Vibrio fischeri*’s bioluminescence.

The second form of analysis was more quantitative as it involved plotting the intensity of the *V. fischeri* bioluminescence as a function of time. While still using the Matlab software, we selected eleven 11×11 pixel regions across the plate: two of which were in the exact locations of the magnets, while the remaining nine areas were chosen based on their proximity to the magnets (whether they were in the magnetic or nonmagnetic region). Refer to Figure 2 for a mapping of the regions on the plate. The intensity of these 11×11 pixel regions was averaged for each section and graphed so that intensity was plotted against time.
Figure 3. A reference for the location of the 11 × 11 pixel areas sampled from the agar plate. Marks indicate selected locations at which the luminescence of the image was studied – see Figures 5-7 below. Red marks represent the points on the agar plate that were directly over the magnets and blue marks represent points that were not over magnets. The magnet on the left was placed so that its positive pole was directed at the plate and the magnet on the right was placed so that its negative pole was directed at the plate. Red marks represent the sections directly over the magnets, and blue marks represent all other areas.

Results

In the course of our experiment, we were able to qualitatively analyze the bacteria’s intensity based on their relevant location to the magnetic fields. Figures 4 and 5 represent the data we collected from two of our trials in which the *V. fischeri* were grown on agar plates, and Figures 6 and 7 represent the data we collected from the two trials in which the *V. fischeri* were grown in photobacterium liquid growth media. For every graph, the red curves represent the intensity of a sampled region on the agar plate directly above the magnets, and the blue curves represent the intensity of various sampled regions dispersed across the plate (See Figure 3 above). We utilized the same procedure for each trial.

An overall trend can be identified between the Figure 4 and 5: at no point in either of the trials did the intensity of the bioluminescent bacteria located directly over the magnets become greater than any other region. While each trial had its own variance with the time the cells were bioluminescent as well as the areas that experienced the most
intense bioluminescence, it can be seen that the red curves did not exhibit the highest intensity at any point during the trials.

However, with Figures 6 and 7 there were variances between the data collected for the liquid culture trials. Figure 6 exhibits a definitive peak in intensity for the area located above the magnet on the plate. Figure 7 exhibits characteristics more similar to those of Figures 4 and 5 because the red curves representing the areas sampled directly above the magnet are not higher in intensity than the blue curves representing other areas of the plate.

![Figure 4](image)

**Figure 4.** Time dependence of luminescence of growth regions in agar plate trial 1. Each curve shows the average luminescence of a different 11x11 pixel region of the agar plate. Eleven separate 11×11 pixel areas were averaged for their intensity; these areas were chosen based on their proximity to the magnetic field generated by the two circular magnets. The two red curves correspond to the areas of the plate sampled directly above the magnets. The remaining blue curves correspond to the nonmagnetic regions on the plate.
Figure 5. Time dependence of luminescence of growth regions in agar plate trial 2. Each curve shows the average luminescence of a different 11x11 pixel region of the agar plate. Eleven separate 11×11 pixel areas were averaged for their intensity; these areas were chosen based on their proximity to the magnetic field generated by the two circular magnets. The two red curves correspond to the areas of the plate sampled directly above the magnets. The remaining blue curves correspond to the nonmagnetic regions on the plate.

Figure 6. Time dependence of luminescence of growth regions in liquid growth media trial 1. Each curve shows the average luminescence of a different 11x11 pixel region of the agar plate. Eleven separate 11×11 pixel areas were averaged for their intensity; these areas were chosen based on their proximity to the magnetic field generated by the two circular magnets. The two red curves correspond to the areas of the plate sampled directly above the magnets. The remaining blue curves correspond to the nonmagnetic regions on the plate.
Figure 7. Time dependence of luminescence of growth regions in liquid growth media trial 2. Each curve shows the average luminescence of a different 11x11 pixel region of the agar plate. Eleven separate 11×11 pixel areas were averaged for their intensity; these areas were chosen based on their proximity to the magnetic field generated by the two circular magnets. The two red curves correspond to the areas of the plate sampled directly above the magnets. The remaining blue curves correspond to the nonmagnetic regions on the plate.

Discussion

In our experiment, we see that there is no indication that a magnetic field influences *Vibrio fischeri*’s quorum sensing-regulated luminescence. Unlike other studies in which the light emission of *V.harveyi* exposed to a magnetic field was twice that compared to the bacteria not exposed to a magnetic field [3], we saw that the variability from one spot to another is far larger than any effect of the magnetic field with *V. fischeri*. Whereas previous studies experimented with magnetic fields ranging from 20 to 2,000 Gauss [3], we experimented with magnets exhibiting a nominally 3,170 Gauss magnetic field. Even with this increased magnetic field, we were unable to verify that a magnetic field had any impact on *V. fischeri* quorum sensing-regulated luminescence in either the agar plate or liquid media trials. From the movies and graphs plotting the intensity vs. time, it is clear that there is no specific deviation from the areas with a direct
magnetic field and those without. Even with trial 1 of the liquid cultures where we saw a peak in intensity for the region of the plate sampled above the magnet, there was only one peak with the red curve instead of two, which indicates that the increase in intensity was not dependent on the magnetic field. Overall, our study indicates that a magnetic field does not have an effect on *Vibrio fischeri* quorum sensing-regulated luminescence due to the variability of intensity from one spot to another in both the solid agar and liquid media plates.

The difference in results could be due to the fact that we used *Vibrio fischeri* in our experiment, whereas other trials employed *Vibrio harveyi*. In order to verify this theory, more experimentation must be done involving different methods. Perhaps the same procedure used to analyze the effects of a magnetic field on the QS of *V. harveyi* [3] can be replicated and applied to *V. fischeri*. Until more investigation is done, the exact effect of a magnetic field on *V. fischeri* quorum sensing-regulated bioluminescence will be unknown.

**Acknowledgements**

I would like to thank Dr. Hagen for his mentorship throughout the summer, as well as Minjun and Delaram for their assistance in the lab. I would also like to thank the Materials Physics REU Program at the University of Florida and the NSF for their DMR-1156737 grant, both of which made this research possible.
References


